# GREEN SYNTHESIS OF SILVER NANOPARTICLES AND EXPLORING THE ANTIOXIDANT, ANTIDIABETIC AND ANTICANCER POTENTIAL OF *Pterocarpus indicus* Willd. STEM BARK AND LEAVES EXTRACTS

*A Thesis Submitted to*

**BHARATHIDASAN UNIVERSITY, TIRUCHIRAPPALLI**

*for the award of the degree of*

**DOCTOR OF PHILOSOPHY IN BIOTECHNOLOGY**

*Submitted by*

**D. VITHYA**

**(Ref. No.18997/Ph.D.K9/Biotechnology/Part Time/November 2018)**

*Under the guidance of*

**Dr. J. SEBASTINRAJ M.Sc., Ph.D.**


Since 1951

**P.G & RESEARCH DEPARTMENT OF BIOTECHNOLOGY**
**JAMAL MOHAMED COLLEGE (Autonomous)**
*College with Potential for Excellence*
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*(Affiliated to Bharathidasan University)*
Tiruchirappalli-620 020, Tamil Nadu, India.

**JANUARY - 2022**

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## *CERTIFICATE*

This is to certify that the thesis entitled **"GREEN SYNTHESIS OF SILVER NANOPARTICLES AND EXPLORING THE ANTIOXIDANT, ANTIDIABETIC AND ANTICANCER POTENTIAL OF *Pterocarpus indicus* Willd. STEM BARK AND LEAVES EXTRACTS"** submitted to Bharathidasan University, Tiruchirappalli for the award of degree of **Doctor of Philosophy in Biotechnology** is an authentic record of original research work carried out by **Ms. D. VITHYA (Ref. No: 8997/Ph.D. K9/Biotechnology/Part Time/November 2018)** under my guidance and supervision during the study period in the P.G and Research Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamil Nadu.

I further certify that this thesis has not previously formed the basis for the award to the candidate, of any Degree / Diploma, Associateship, Fellowship or other similar titles. The thesis as a whole is the independent work of the candidate.

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## *DECLARATION*

I hereby declare that the thesis entitled **"GREEN SYNTHESIS OF SILVER NANOPARTICLES AND EXPLORING THE ANTIOXIDANT, ANTIDIABETIC AND ANTICANCER POTENTIAL OF *Pterocarpus indicus* Willd. STEM BARK AND LEAVES EXTRACTS"** which I submit for the award of the degree of **Doctor of Philosophy** in the Bharathidasan University is the original work carried out by me under the guidance and supervision of **Dr. J. Sebastinraj**, Assistant Professor, Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli – 620 020.

I further declare that this work has not been submitted earlier in full or in parts to any other university for the award of any other degree or diploma.

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Date:

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This thesis is dedicated to

my parents.

For their love, support and

encouragement

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**(D. VITHYA)**

# CONTENTS

# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| µl | – | microlitre |
| µm | – | Micrometre |
| 3D | – | Three Dimensions |
| 3ERT | – | Human Estrogen Receptor |
| ADME | – | Absorbance, Metabolism, excretion and Distribution |
| Ag | – | Silver |
| AgNPs | – | Silver Nanoparticles |
| ANOVA | – | Analysis of variance |
| DMEM | – | Dulbecco's Modified Eagle's Medium |
| DMSO | – | Dimethyl sulfoxide |
| DNS | – | Dinitro salicylic acid |
| DPPH | – | 2,2-diphenyl-1-picryl-hydrazyl-hydrate |
| ER | – | Estrogen Receptor |
| FBS | – | Fetal Bovine Serum |
| FTIR | – | Fourier Transform Infra Red Spectroscopy |
| GC-MS | – | Gas Chromatography Mass Spectrometry |
| GI | – | Gastrointestinal |
| MCF- 7 | – | Human Breast Adenocarcinoma Cell Line |
| MTT | – | 3-(4,5 -di-methylthiazol-2-yl)-2,5-diphenyl-2H-trtra-zolium bromide |
| mV | – | millivolt |
| Nm | – | Nanometre |
| PBS | – | Phosphate Buffered Saline |
| PDB | – | Protein Data Bank |
| pNPG | – | P-nitro phenyl glucopyranoside |
| RCSB | – | Research Collaboratory for Structural Bioinformatics |
| SEM | – | Scanning Electron Microscope |
| SPSS | – | Statistical Package for the Social Sciences |
| TCA | – | Trichloroacetic acid |
| TM | – | Tamoxifen |
| XRD | – | X-ray diffraction |
| α | – | alpha |

# LIST OF FIGURES

# LIST OF TABLES

## LIST OF CHARTS

# 1. Introduction

## 1.1 Nanotechnology

Nanotechnology refers to combination or mixing of two words namely Greek word nano refers to a billionth and the word technology. Nanotechnology concept has been introduced before the ninth century and it is considered to be a modern medicine. A Mesopotamia artisan was the first to use the gold and silver nanoparticles to produce glittering properties on pots. So, nanoparticles are found to be a size below 100 nm or 100 μm (Jack, 2013). Nanoscience deals about the properties and materials response at macromolecular scale, atomic and molecules and generally size varies between 1-100 nm. Nanotechnology can be used for industrial purpose but also focused in medical technology and meteorology. Nanotechnology is an upcoming field where 50,000 articles on nanotechnology have been published worldwide annually. In the present situation, there is a need and continuous way of exploration and development of cheaper, effective new plant-based nanoparticles with better bio active component and least side effects (Arumugam Manthiram, 2019).

## 1.2 Nanotechnology - Modern Medical Science

Allopathic medicines are also known as modern medicine they are based on scientific approach to treat and diagnose the disease and made an unbelievable advance in current trend. It can be used in emergency situations like life threatening problems, accidents, and trauma by performing radiation therapy, chemotherapy, steroids, antibiotics and surgery. The major drawback in modern medicine is it does not concentrate on the root of problematic diseased condition instead it treats only for diseased symptoms.

After consuming modern medicine, it produces a late reaction including several allergies and more over many side effects. In case of alternative medicine, it is designed based on the condition of body and mind and it is also a cost effective and plays an important role in curing chronic illness without any side effects (Eric Drexler, 1986, 1992).

## 1.3 Nanomedicine

Nanomedicine research is multidisciplinary field they mainly use nanomaterials for designing nanomedicine. The main goal is based on drug delivery is targeted to provide the medication to diseased part of the body so that drug can accumulate the diseased area. Nanomaterials are designed in different size to carry that drug molecule. The main concept is that the nanoparticles surface depending upon this property the drug can be placed in the body with increase in circulation time (Ahmad *et al.*, 2019).

## 1.4 Nanoparticles

Nanoparticles also known as nanocrystals they are obtained from semiconductors, oxides and metals. They have been used for variety of applications especially for commercial purpose and they are being selected based on chemical, mechanical, magnetic, electrical and optical properties. Nanoparticles can be defined as; a tiny particle consist of small matter composed to be made up of whole unit based on properties and transport. Nanoparticles are colloidal solid particles and consist of group of atoms and their size varies from 1-100nm. They made up of large number of macromolecule materials because of these properties they can be used in therapeutic field as drug delivery and as a carrier in vaccines. The nanoparticles can be encapsulated, entrapped, dissolved, and chemically attached. Nanoparticles can also be made from polymers and they are based on natural and synthetic.

There are two types of nanoparticles and they are classified based on preparation properties such as nano capsules and nanospheres. Nano capsules are wall like structure with a membrane in the exterior portion were only the drugs are encapsulated in the absorbed form. Nanospheres are matrix like structures (monolithic type) drugs are absorbed on their surfaces. It is difficult to explain whether the nanoparticles are membrane or matrix type. Nanoparticles synthesized based on biological method from plant extracts show enzyme kinetics for catalytically activity, offers better manipulation, crystal growth can be controlled and also maintain stability.

In research field, nanoparticles are promising upcoming field using minute size of the particles which has wide range applications. The most prominent nano product is nano silver because of its biological properties which are essentials for consumers like food and medicinal applications especially in diagnosis, drug delivery and imaging. Because of the small sized nanoparticles which have different properties when compared with bulk materials. Nanoparticles possess small size but large surface area when compared with volume (Lee, *et al.*, 2012).

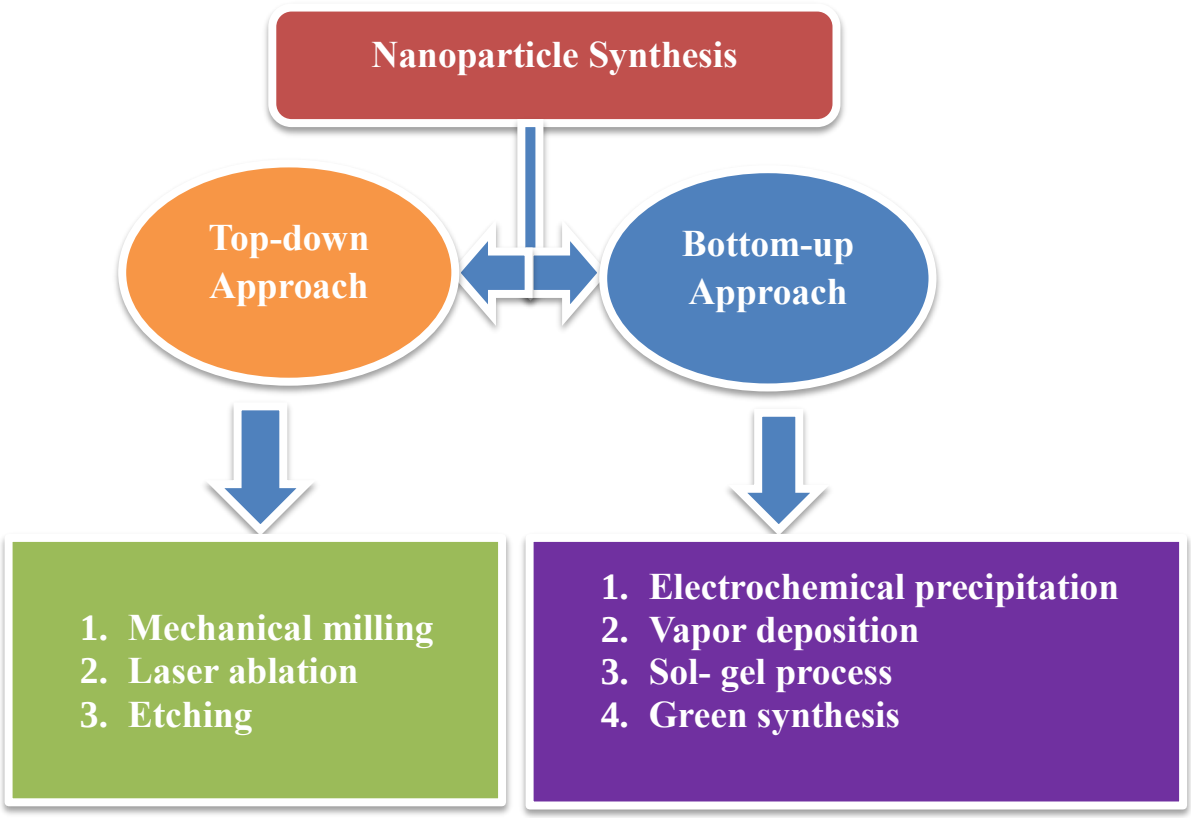
### 1.4.1 Nanoparticles Effect on Plant Growth and Development

Depending upon the properties of nanoparticles when they interact with plants, they produce many morphological and psychological changes. Nanoparticle's efficacy can be determined by their chemical composition, size of nanoparticles, surface covering, reactivity of particles dose at which they are effective. There is both positive and negative effect on plant growth and development and engineered nanoparticles have high impact upon plants and also upon their concentration, composition, size, physical and chemical properties of designed nanoparticles moreover as plant species. Nanoparticle's efficacy may vary from plants to plants.

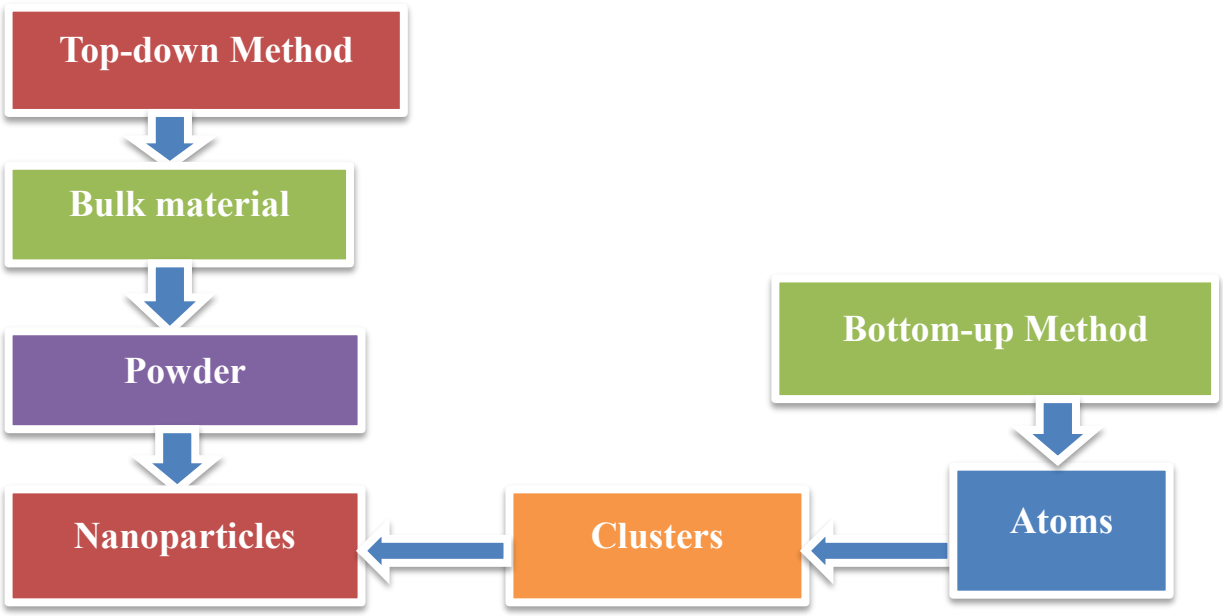
### 1.4.2 Synthesis of Nanoparticles

There are many chemical and green ways to synthesize nanoparticles such as solid reaction, co-precipitation, chemical reaction, sol gel method, microwave radiation. Nanoparticles because of their size dependent features there are very essential in several natural associated activities and human welfare. The size of the particles less than 100nm diameter are not usually present on the earth so their presence is due to photochemical volcanic activity, food cooking and vehicle exhausts, but Prof. Norio Taniguchi in 1974 introduced the research technology from physics, chemistry and nano technology. The noble metals are especially silver, gold, aluminium, platinum, palladium, copper, zinc and iron were used in the synthesis of nanoparticles because of their nano size. There are some physical and chemical methods which are based on conventional so they are considered to be hazardous and expensive.

The nanoparticles which are obtained from biological based method show high stability, high yield, and solubility. There are many methods in which nanoparticles can be synthesized. Nanoparticles synthesized by synthetic method are high hazardous so the best method to synthesize nanoparticles is biological method which are green, dependable, non-toxic, rapid and simple and produce high morphology well defined size and the nanoparticles synthesized from green procedure are supposed to be a promising one (Kaviya *et al.*, 2011, Hashim, 2012 and Khalil *et al.*, 2013).



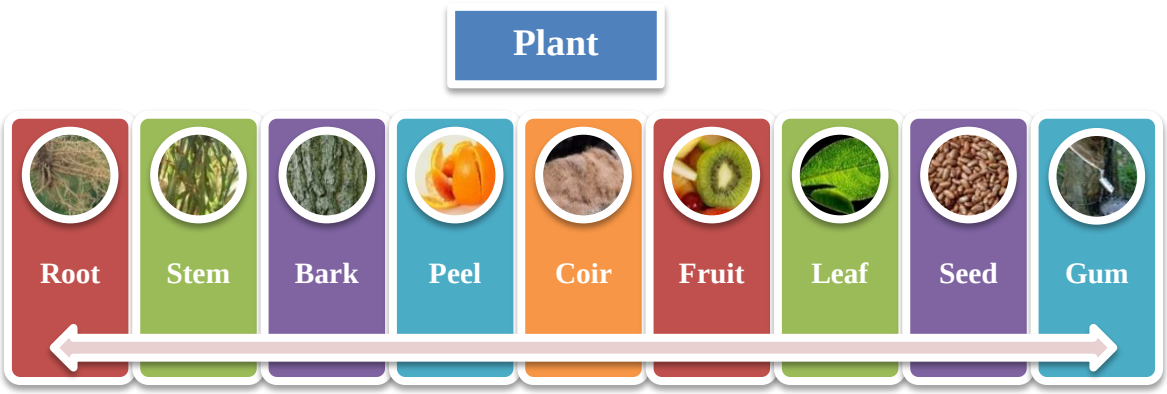

**Schematic Representation of Nanoparticle Synthesis**



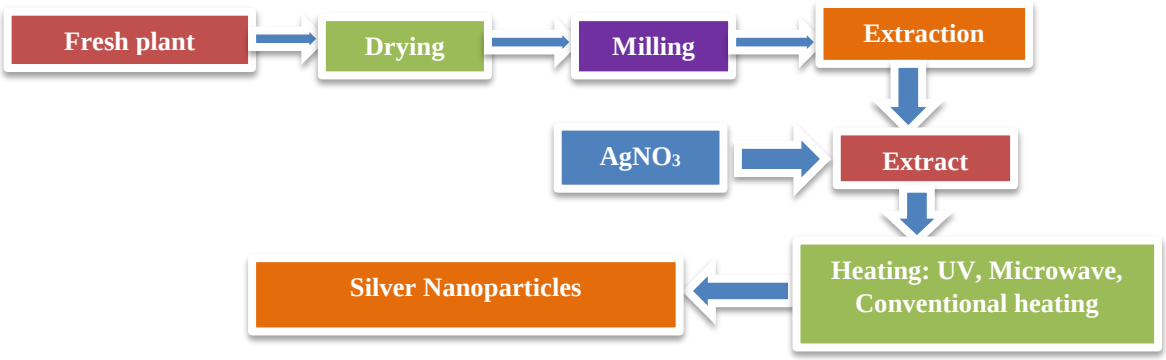

**Process of Nanoparticles Synthesis**

### 1.4.3 Green Synthesis of Nanoparticles

The factors such as time and energy are consumed and the synthesis takes place at high temperature and pressure in physical method. Synthesis takes at low temperature and even inexpensive, the use of toxic reducing and stabilizing agents makes it harmful in chemical method. Green synthesis of nanoparticles is an easy, efficient, non- toxic and eco-friendly method. It consumes low energy and produces safer products and byproducts (Mittal *et al.*, 2014). In green synthesis, the nanoparticles are synthesized with plant extract through reduction process. Approach of green chemistry is to prevent pollution.




**Synthesis of AgNPs involves different parts of Plant**




**Schematic representation of Green Synthesis of Nanoparticles**

### 1.4.4 Advantage of using Green Synthesis

Chemical synthesis of nanoparticles may lead to some toxic chemicals adsorbed on the surface that may lead to some adverse effect in the medical application. Alternative to chemical method green synthesis is cost effective, eco-friendly and easily scaled for large scale synthesis and there is no need of using toxic chemicals, high pressure, energy and temperature. Nanoparticles are classified based on organic, inorganic, metallic, and semiconductor nanoparticles due to their primary material properties (Zhang *et al.*, 2016).

### 1.4.5 Applications of Nanoparticles

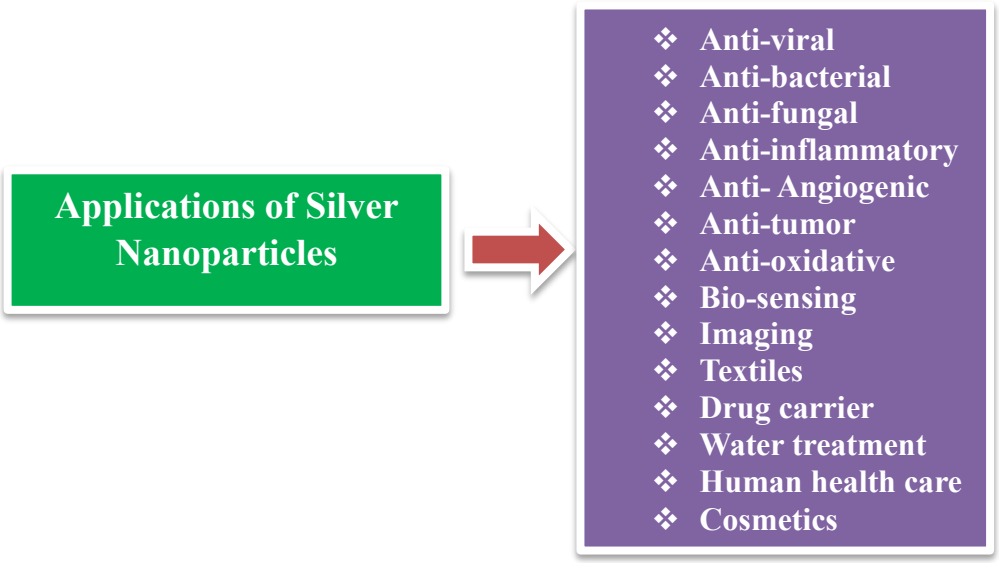
Nanoparticles are used in broad range of applications. The size and structure of nanoparticles are similar to biological molecule therefore nanoparticles can be used for research. Nanoparticles are widely used in science field that results in the development of research in medical field, analysis of drugs in delivery, device diagnosis and analytical trots. There are plenty of nanoparticles such as organic, inorganic, nanocrystals, nanotubes, polymeric structures such as dendrimers. Because of their wide variety of applications, they are primarily used in research and drug delivery system (Nayak *et al.*, 2010). Current trend is based on herbal nanomedicine which is becoming a frontier in the nanoformulation research field. Recent scientific research proves that herbal nanomedicine is very much useful for the treatment of cardiovascular diseases, diabetes, and anti-osteoporosis. The main advantage of nanotechnology in medical field is that tiny devices can be possibly inserted inside the body and these drugs are more efficient than synthetic drug (Chugh *et al.,* 2018).

## 1.5 Silver Nanoparticles

There are several noble metal nanoparticles but silver nanoparticles have been attracted because of their unique properties like chemical stability, electrical conductivity and catalytic activities when compared with bulk materials. Silver in nanoscale region has different properties from the same materials because of their high surface to volume ratio (Rogers *et al.*, 2008). For the nanoparticles synthesis temperature is one of the important factors which increase the reaction rate and its efficiency. Nucleation rate can be elevated by increase in temperature. Silver nanoparticles have wide range of biomedical applications. Synthesis process involves eco- friendly procedure for large scale production and it is said to be highly stable in nature (Stern, 1972).

### 1.5.1 Application of Silver Nanoparticles

Silver nanoparticles are applied in various field including medicine, industries, health care, food materials because of their unique physical and chemical properties. Because of rare and peculiar properties of silver nanoparticles they are used as antibacterial agents, device coatings in medicines, cosmetics, pharmaceuticals, food industry, orthopaedics, anti-cancer drug which enhances the effect of killing tumour cells. In the current situation, silver nanoparticles are used in biomedical system, dressing of wounds and textile shop. Because of their unique surface to volume ratio properties, they can change the physical, biological, metallic and chemical properties (Kim *et al.*, 2007 and Keat *et al.*, 2015).




**Various Application of Silver Nanoparticles**

## 1.6 Diabetes Mellitus

Diabetes mellitus is a universal endocrine disorder. Due to insulin resistance or deficiency leads to high sugar level in blood for a long period of time is referred to as diabetes mellitus. The glucose level gets raised in blood during pregnancy period is referred to as gestational diabetes. Certain anti-diabetic medications and insulin are available for the prevention of this disorder (Nadaroglu *et al.*, 2017).

## 1.7 Diabetes Mellitus and Breast Cancer

Diabetes mellitus and breast cancer are most prevalent chronic diseases among women (Torre *et al.*, 2015). Approximately 16% of breast cancer patients suffered from diabetes (Corriere *et al.*, 2013). Diabetes mellitus has been identified as an independent risk factor for breast cancer (Guariguata *et al.*, 2014). A well- designed meta-analysis suggested that the women with diabetes had a 23% greater risk of subsequent breast cancer (De Bruijn *et al.*, 2013). The pre-existing diabetes is associated with a 37% greater risk of breast cancer mortality in female breast cancer patients, respectively (Zhou *et al.*, 2015).

Coughlin *et al.,* 2004 followed 1.2 million US men and women in all states, the district of Columbia from 1982 to 1988 and found that, women with diabetes at baseline were more likely to die from breast cancer than were women not diagnosed with diabetes. Similarly, Verlato *et al.*, 2003 followed a cohort of 3782 diabetic women in northern Italy from 1987 to 1996 and observed a higher risk of dying from breast cancer.

## 1.8 Anti-Breast Cancer Activity of Silver Nanoparticles

Breast cancer is the leading cancer which causes death mostly among women, it is considered as a second largest death causing cancer in united states. In 2013 American cancer society reported a survey, about 39,620 women leading to death due to breast cancer and 2,32,340 women are expected to have breast cancer (Alteri *et al.*, 2013). The current cytotoxic agents worn for the breast cancer treatment are highly expensive and not efficient because of their side effects due to the toxic nature of the non-cancerous tissue (Kim *et al.*, 2007 and Yeruva, *et al.*, 2008). Today the most efficient nanoparticles used to treat cancer cells are the silver nano particles, it is a biologically synthesised active nanoparticle against human breast cancer (Gurunathan *et al.*, 2013 and Vivek, *et al.*, 2012). Breast cancer arises from breast tissue, most frequently from the milk ducts inner line or from the lobules. Breast cancer most commonly occur in women than in men (Krishnaraj *et al.*, 2010).

## 1.9 The use of Silver Nanoparticles as Antioxidant Agent

AgNPs showed greater antioxidant activity comparable to that of ascorbic acid. The phytochemicals (flavonoids) and silver ions could serve as antioxidants through single electron and hydrogen atom transfer (Wang *et al.*, 2018).

According to (Johnson *et al.*, 2018), the antioxidant ability of AgNPs is caused by the presence of phenolic compounds, terpenoids, and flavonoids in plants which allow nanoparticles to act as singlet oxygen quenchers, hydrogen donors, and reducing agents. The DPPH and ABTS radical scavenging abilities were dose-dependent, which means that increasing scavenging activities against both radicals with the increasing concentration of AgNPs were observed (for DPPH and ABTS - 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) the antioxidant activity was in the range 62.2–82.4% and 64.5–96.8%, respectively (Docea *et al.*, 2020).

### 1.10 *In Silico* Molecular Docking Studies for Diabetes Mellitus

Diabetes mellitus is chronic hyperglycemia or high blood glucose with interference of the metabolism of fats, proteins and carbohydrate that ends from deficiencies of insulin excretion and insulin activity (Nair *et al.*, 2013). The two most common types of diabetes are type 1 diabetes mellitus with about 5-10 % and type 2 diabetes mellitus with about 90-95% of total patients (Yee and Fong, 1996). Alpha-amylase is related to the breakdown of carbohydrate and alpha-glucosidase is associated with the breakdown of starches and disaccharide into monosaccharide glucose (Van de Laar, 2008). Both alpha-amylase and alpha-glucosidase inhibitors acarbose are oral antidiabetic drugs used for controlling carbohydrates digestion that are converted into sugars and absorbed by the intestines (Kitchen *et al.*, 2004). Natural products identified from the medicinal plants are used to reduce the blood sugar levels and to maintain blood sugar homeostasis with fewer side effects is becoming more important for the treatment of diabetes. Recent computational or molecular docking analysis could be an easy gate-way for predicting effective drugs of natural compounds against diabetes mellitus (Gilson and Zhou, 2007).

However, in silico docking of the enzymes alpha amylase and alpha glucosidase were carried with the national compounds from the medicinal plants to analysis their potency (Qing *et al.,* 2014).

### 1.11 *In Silico* Molecular Docking Studies for Breast Cancer

Breast cancer is the serious health problem in India causing the highest mortality rate in females, which occur due to the uncontrolled cell division and can be spread to other parts of the human body (Jemal *et al.*, 2011). The prevalence rate of the breast cancer in India is more than 90,000 in the coming years and over 50,000 women die each year (Chi *et al.*, 2013). The patients with 60% breast cancers are diagnosed as estrogen receptor alpha positive (ERα) cancers. The estrogen receptor alpha is mainly responsible for the breast cancer development with regulation of the transcription of various genes as a transcription factor (Tan *et al.,* 2009). Both estrogen receptor alpha and beta are naturally present in human population and is mainly expressed in the mammary gland and uterus. In women, estrogen receptor plays an important role in inflammation, maturation, differentiation, apoptosis and proliferation of breast cancer (Bai and Gust, 2009).

The drugs Tamoxifen, Raloxifene and Toremifene used for the treatments of breast cancer which causes more side effects such as strokes, blood clots, uterine cancer or cataracts (Parkkari *et al.,* 2003 and Freedman *et al.,* 2011). *In silico* analysis was carried out to analyze docking against the estrogen protein of breast cancer utilizing virtual screening methods (Sastry *et al.*, 2010). This virtual screening method is based on computational analysis widely used to identify the structure, drug target and the analysis of protein receptor complex (Marquette and Nabell, 2012). The molecular docking method is useful in the identification of suitable site of receptor protein. The interaction energy of ligand – receptor was obtained after docking the ligands with active site

(3ERT) (Jiang *et al.*, 2006). The natural compounds from the medicinal plants which are also having binding affinity for breast cancer receptors could lead to breast cancer treatment by docking method.

## 1.12 Medicinal Plants - An Alternative Source

A new drug discovery provides a variety source because of its nature since it derived from medicinal plants for treating multi drug resistant infections. Secondary metabolites for treating like poly phenols, flavonoid, glycosides, saponins, triterpenes, alkaloids, etc., produced by plants are pharmacologically active. The advantage of using natural products is therapeutically safe, economically friendly and lesser side effects. Combination therapy is the combination or singly plant extracts with antibiotics or other plant extracts or other chemicals. The development of novel ways of fighting the drug resistance mechanism proves to be a good situation against new microbes. A medicinal plant with biologically active polyphenolic compounds isolated from plant species contains antioxidant, anti-microbial, and nutraceutical properties. Plant materials can also be used as an alternative as nanofabricators which promote green synthesis is less expensive and less toxic. The poly phenols, microbes and natural biomolecules for the synthesis of nanoparticles from plants are considered as the best substitute for physical and chemical method for eco-friendly nature (Vlietinck, 1999 and Heinrich *et al.*, 2017).

## 1.13 *Pterocarpus indicus* Willd.

*Pterocarpus indicus* Willd is plant belonging to the family Fabaceae and it is also recommended as an ornamental avenue tree and the reddish hard wood is an excellent timber in Southern Asia. A red, gum like resin from the bark is used in folk remedies for tumours and the leaves for cancer, especially of mouth cancer (Wang *et al.*, 1997).

### 1.13.1 Distribution

*Pterocarpus indicus* is widely distributed over tropical and subtropical South Asia as India, Malaysia, Brunei, Thailand and Indonesia (Ragasa *et al.*, 2005).

### 1.13.2 Systematic Position

| | |
|---|---|
| **Kingdom** | Plantae |
| **Order** | Fabales |
| **Family** | Fabaceae |
| **Subfamily** | Faboideae |
| **Tribe** | Dalbergieae |
| **Genus** | *Pterocarpus* |
| **Species** | *P. indicus* |

**1.13.3 Vernacular/Common Names:** Vengai, Red sandalwood, narra, rosewood, Burmese rosewood (trade names), malay paduak (English), sonokembang (Indonesia), Sena (Malaysia and Singapore), prickly narra and smooth narra (Philippines).

### 1.13.4 Chemical Constituents

- *Pterocarpus indicus* shown to produce phytochemical classes including isoflavonoids, aurones, lignans, flavonoids, lupeol, stilbenes, triterpenes, sesquiterpenes, sterols.
- Wood contains the red colouring matter santalin and narrin and angolensin.
- Narrin is a dark red powder which yields resorcinol and phloroglucinol on fusion with alkali.

### 1.13.5 Pharmacological Activities

- The health benefits and potential biological activities of *Pterocarpus indicus* including antidiabetic, antimicrobial, antioxidant, cytotoxic activities, hepatoprotective, anti-inflammatory properties and protective effects on the liver, nervous system and gastric mucosa.
- *Pterocarpus indicus* are used in the treatment of various diseases such as diarrhoea, fever, urinary tract and skin infection, control blood sugar and toothache.
- The heartwood of some *Pterocarpus* species have been reported as anthelmintic, clear jaundice, relieve ulcer, cure elephantiasis, rectalgia, leucoderma, greyness of hair and cough.
- The bark and resin decoction of many *Pterocarpus* species are used for the treatment of urethral discharge, gland tumours, chronic ulcer and ringworm of the scalp (Khan and Omoloso, 2003; Mankani *et al.*, 2005; Tippani *et al.*, 2010; Anowi *et al.*, 2012 and Umeh *et al.*, 2014).

## 1.14 Objectives

The present study is mainly focussed with the following objectives.

- The *Pterocarpus indicus* Willd. plant leaves and stem bark was collected from Senthankudi Village, Pudukkottai District, Tamilnadu, India, and it was identified and authenticated by Department of Botany, St. Joseph's College, Trichy.
- Qualitative and Quantitative estimation of phytocompounds in the bark and leaves of *P. indicus* and to analyze the secondary metabolites using GC – MS.
- To synthesize silver nanoparticles (AgNPs) by using ethanolic bark and leaves extract of *P. indicus*.

- To characterize *P. indicus* bark and leaves mediated AgNPs using different techniques such as UV – Vis, FTIR, SEM, XRD, Particle Size and Zeta Potential.
- To study Antioxidant potential of *P. indicus* bark and leaves mediated AgNPs using DPPH assay and Reducing power method.
- To study Antidiabetic activity of *P. indicus* bark and leaves mediated AgNPs using alpha-amylase and alpha glucosidase inhibitory assay.
- To study the anticancer potential of *P. indicus* bark and leaves mediated AgNPs on human breast cancer cell line, MCF – 7.
- *In Silico* molecular docking analysis of bioactive compounds present in bark and leaves of *P. indicus* for alpha-amylase and alpha glucosidase inhibitory assay.
- *In Silico* molecular docking analysis of bioactive compounds present in bark and leaves of *P. indicus* for Estrogen Receptor.

# 2. Review of Literature

## 2.1 Nanoscience

The Greek word nano in nanotechnology is the measurement of one billionth unit. The nanosecond and nanometre referred to one billionth of a second and a meter respectively. The invisible objects in this world cannot be seen by the naked eye. The atom spacing in the matter illustrated the concept which is about 1/10 ($10^{-1}$) nm, 2nm wide is a strand of DNA and 2,000,000,000 ($2 \times 10^{9}$) is the height of a six-foot person. Nanoscience technology is widely used in the field of Physics and Chemistry. In the field engineering, medicine and biology, it plays a major role. The physical and chemical synthesis tend to be more labour-intensive and also hazardous than the biological synthesis (Prasad *et al.*, 2014).

## 2.2 Properties of Nanoparticles

Nanoparticles can be categorized as organic and inorganic nanoparticles. Organic nanoparticles incorporate carbon NPs like quantum dots, carbon nanotubes and fullerenes. Inorganic nanoparticles incorporate semi-conductor NPs like ZnS, ZnO, CdS. Metal NPs like Au, Ag, Cu, Al and magnetic NPs like Co, Ni, and Fe (Parashar *et al.*, 2009). There is a growing enthusiasm for silver nanoparticles and zinc nanoparticles as they furnish characteristics with useful applications. Nanotechnology plays a vital in performance on many fields of technologies such as Information technology, homeland security, medicine, transportation, energy, food safety and environmental technology etc. (Lee *et al.*, 2007; Morley *et al.*, 2007; Kudle *et al.*, 2014).

The solid particles with a size of 10-1000nm will come under the nanoparticles (Ibrahim *et al.*, 2015). There is wide application of nanoparticles in the field of biomedical, optical and electronic. Nanoparticles serves as a connecting material between the top-down which allows the bulk material to break and bottom-up approach breaks the structure of atoms or molecules (Hyllested *et al.*, 2015).

## 2.3 Green Synthesis of Silver Nanoparticles

The Green synthesis of nanoparticles is an easy, efficient, non- toxic and eco-friendly method. It takes low energy and produces safer products and by-products (Rai *et al.*, 2009). In green synthesis, the nanoparticles synthesized with plant extract through reduction process. Green synthesis of silver nanoparticles has many medicinal applications such as optical property, antibacterial effects, sensor, optical probes and also reported to have a better antidiabetic activity (Rout *et al.*, 2012).

## 2.4 Synthesis and Characterization of Silver Nanoparticles

The silver nanoparticles synthesized using *Capsicum annuum was* confirmed by UV, FTIR, XPS, XRD and TEM. *Jatropa curcas* leaf extract has effective capping and reducing agent for AgNPs synthesis which was analysed by UV, XRD, and HRTEM. The adsorption of UV is 425nm and SAED patterns is displayed by HRTEM images. The silver nanoparticles synthesis was done by the leaf extract of *Eucalyptus hybrid* and were analysed by UV, SEM, XRD and EDX. The plasma resonance peak of UV is 412nm. The nanoparticle is crystalline in nature that was analyzed using XRD. The AgNPs shape is uniform and that was confirmed by SEM. The green nano synthesis is eco-friendly compared to chemical and physical method (Li *et al.,* 2007; Bar, *et al.*, 2009; Dubey *et al.,* 2009)

*Foeniculum vulgare* plant extract is used for nanoparticle synthesis and analysed by UV and NTA. The plasma resonance of UV spectrum is 427nm. The distribution of AgNPs is the particle size is LM-20. This plant is active against *Staphylococcus aureus* and *Escherichia coli*. The silver nanoparticles synthesized by ethyl acetate extract from the plant *Ulva fasciata* is active against *Xanthomonas campestris* and *Mlavacearum*. It was analysed by SEM, XRD, FTIR, UV and EDX. Plasmon resonance shows brown yellow colour at 440nm in UV spectroscopy. Reducing and stabilizing agent are hexadecenoic acid and 1-(Hydroxymethyl)-2,5,5,8A-tetramethyl decahydro-2-napthalenol (Bonde *et al.*, 2011 and Rajesh *et al.*, 2012).

The plant *Panicum virgatum* is commonly known as switch grass synthesised silver nanoparticles in an ambient temperature and it was characterized by UV XRD and TEM. XRD shows crystalline of AgNPs in lattice parameter of $4.0962A^0$. The UV peak is 435nm. The TEM shows that the size ranges from 20-40nm. The green synthesis of silver nano particles was done using *Aspergillus terreus*is and analysed by UV, XRD and TEM. The silver nanoparticles extracted from *Myrica esculenta* leaf was analysed by UV, XRD and TEM. The absorption peak of UV is 466nm, TEM shows the size as 23nm. (Mason *et al.*, 2012; Li, *et al.*, 2012; Phanjom *et al.*, 2012).

Vanaja *et al.*, (2013) used the *Coleus aromaticus* leaf extract for the bioreduction of silver ions to silver nanoparticles. The factors such as 1 mM silver ion concentration, pH 8.2 and temperature 70 ºC is more favourable for maximum production of silver nanoparticles. UV-visible spectrum confirmed the formation of silver nanoparticles and the SPR band was observed at 460 nm indicates particles are poly dispersed.

Iravani *et al.*, (2013) studied about the green synthesis of silver nanoparticles with *Pinus edarica* bark extract. UV-Visible and TEM method were used to characterize the

formed silver nanoparticles. The synthesis of AgNPs is completely based on the quantity of extract, substrate concentration, temperature and pH. UV-Visible spectrum showed absorption peak at 430nm. TEM confirmed the particle size of AgNPs in the range of 10-40nm which is spherical in shape. This study reported that the green synthesis is cost – effective and environment friendly.

The silver nanoparticles extracted from *Olive leaf* showed anti-microbial activity. The adsorption peak is showed by UV, XRD, SEM, TGA the final range is 440-458nm. Synthesis is based on different concentrations, they are (0.2,0.5,3.5,7ml). The antibacterial activity was studied based on pH, contact time, temperature, reaction rate and bacterial activity and it is helpful for therapeutic approach. The aqueous flower extract of *Bougainvillea spectabilis* was used for the synthesis of silver nanoparticles. Synthesis and formation of silver nanoparticles were confirmed by color change from pink color to brown color and it was further characterized by ultraviolet (UV) visible spectroscopy at the range of 300 to 800 nm. The peak showed at 431 nm. Further morphology, shape and size of the synthesized nanoparticles were characterized by field emission electron microscopy and the presence of metal silver was analyzed by energy dispersive x-ray spectroscopy (Khalil *et al.*,2014 and Bharathi *et al.,* 2016).

The green synthesized silver nanoparticles using *Ficus racemosa* bark exhibit antimicrobial activity. UV-visible, FTIR, SEM and EDAX were characterized for this study. The author confirmed that this protocol as simple, rapid, one step eco-friendly, non-toxic and an alternative conventional Physio-chemical method. The biosynthesis of silver nanoparticles in an alternative way with *Raphanus sativus.* UV-Vis, SEM, XRD and DLS were used for the characterization of synthesized silver nanoparticles. UV-Visible spectrum observed surface Plasmon resonance at 430nm. DLS analyses confirmed the particle size in the range of 34-50nm. This study revealed that the synthesis

of AgNPs occurs at 50° C and reported that the green synthesis is cost-effective and environment-friendly. The gold nanoparticles of *Terminalia chebula* fruit extract was characterized using UV, XRD, and SEM with EDAX. Synthesized silver nanoparticles have found to be a potential one (Priya *et al.*, 2016; Fadel *et al.*, 2017; Rose *et al.*, 2019).

### 2.5 Application of Silver Nanoparticles using Medicinal Plants

The silver nanoparticles of *Argemone mexicana* leaf exhibit antimicrobial activities. They were analysed by UV, XRD, FTIR and SEM. The SEM is analysed by range of 25-50nm.The disc diffusion method was done against *Escherichia coli, Pseudomonas aeruginosa and Aspergillus flavus.* The silver nanoparticles of *Cantharanthus roseus* leaves were synthesized and exhibit antimicrobial activities. They were characterized by the UV, SEM, EDX, and XRD. The analysis of SEM range is 35-55nm. It shows anti-plasmodial activity and also helpful for nanotechnology and biomedical activity (Singh *et al.*, 2010 and Ponaruselvam *et al.*, 2012).

The gallic acid biased silver nanoparticles and anti-phytopathogenic activity with *Terminalia chebula* was reviewed and the synthesized nanoparticles were characterized using UV- Vis, Powder XRD, TEM and SEM. UV-Visible spectrum showed absorption peak at 426nm. TEM showed the particle size in the range of 14-60nm. The author concluded that the synthesized silver nanoparticles were found to possess anti-phytopathogenic activity against *Xanthomonas axonopodis pv. malvacearum* by broth microdilution method (70µg/ml) (Sulthana *et al.*, 2014).

The author studied the synthesized silver nanoparticles and their antibacterial effect of *Terminalia chebula* fruit extract. The synthesized nanoparticles were characterized using UV-Vis, FTIR, and SEM. It was found that synthesized silver nanoparticles of *Terminalia chebula* show more resistant against all microbes tested

when compared with methanol and aqueous extracts. The silver nanoparticles using bark of *Pongamia pinnata* shows antibacterial activity against certain pathogens. UV- Vis, X-ray diffraction, SEM characterization were used to confirm the silver nanoparticles (Prathiba *et al.,* 2015 and Rajesh Kumar, 2016).

Espenti *et al.,* (2016) reviewed the synthesis of silver nanoparticles biologically with *Syzygium cumini* and their antimicrobial potential. The synthesized AgNPs were characterized by XRD, FTIR, DLS and Zeta potential analysis. UV-Visible spectrum observed the absorption peak at 420nm at basic pH of 9.0 (complete nucleation and increased growth of AgNPs). TEM confirmed that AgNPs are smaller in size, monodispersed within the range of 15nm. Biosynthesized silver Nanoparticles showed the inhibition against pathogens in humans and added an herbal value in biomedical and nanotechnology industries.

Rana *et al.,* (2016) studied about green synthesis of ZnO nano sized spherical nanoparticles with *Terminalia chebula* fruit extract. XRD, FTIR, FESEM, UV and photoluminescence spectroscopy and EDAX were analyzed. Synthesized ZnONPs are stable, hexagonal structure, roughly spherical in shape. PL spectra found low intensity near band emission peak at 396nm. ZnO nanoparticles were used as a potential semiconductor of photo catalyst in waste water treatment which degrades Rhodamine B (RhB) effectively.

Ali *et al.,* (2016) suggested the green synthesis of silver nanoparticles with Apple and evaluated its antibacterial activity. Silver nanoparticles are characterized using UV, FTIR, XRD, and Zeta potential. FTIR analyses identified the ethylene groups/residues and capping agent. DLS analyses showed the average size of 30.25± 5.26nm and zeta

potential found at 5.68±3.28mv. This study revealed that the synthesized AgNPs showed minimum bactericidal concentration (MBC) at 125-1000µg/ml.

Majeed and Khanday (2016) reported the green synthesis of silver nanoparticles with bark extract of *Salix alba* and evaluated the antimicrobial effect isolated from Dental plaque. The synthesized silver nanoparticles were characterized by UV, Zeta potential, AFM, and TEM method. UV-Visible spectrum showed absorption peak at 440nm. HRTEM images recorded the particle size in the range of 29-35nm. Atomic force microscopy observed the particle size in the range of 30-50nm and is highly stable. The study found that the synthesized silver nanoparticles showed a good antibacterial activity against several bacterial pathogens and animal trial is required for further approach.

Khan *et al.,* (2017) analyzed the novel synthesis of silver nanoparticles with aqueous leaf extract of *Aloe vera* and evaluated their antimicrobial activity. UV, FTIR, SEM, and EDX techniques were analyzed under this study. Synthesized silver nanoparticles have a strong efficacy against *S. aureus* (04mm, 09mm, 13mm, 17mm and 19mm) using gentamicin as standard drug except *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.*

Rose *et al.,* (2017) studied that the aqueous extract of *Sansevieri atrifasciata* by using copper nanoparticle synthesis of *Escherichia Coli and Staphylococcus aureus* and it also studied by analysis of copper nanoparticles by using biologically and chemically in antibacterial activity. It is characterised by UV, XRD, EDX, SEM. The plasma resonance of UV peak is 585nm it is highly stable. The average range of SEM is 29-68nm.The antibacterial activity has better than the stability of synthesized by CuNPs.

Arshad *et al.,* (2018) studied about the green synthesis of silver nanoparticles with bark of *Ailanthus actissima.* UV-Vis, FTIR, SEM, and DLS technique is used to

characterize the silver nanoparticles. UV-Visible spectrum showed intensity peak and shape of spectrum based on the ratios. SEM and DLS showed the average size of 80nm. This study found that the synthesized silver nanoparticles showed more effective activity against antibacterial and antifungal agent when compared with other extracts.

Moodley *et al.,* (2018) analyzed the green synthesis of Ag nanoparticles with *Moringa oliefera* leaf extracts and showed significant antimicrobial activity. UV-Visible spectrum showed absorption peak at 4550nm for fresh leaf extracts and 440nm for freeze-dried leaf extracts. This study showed broad spectrum of antimicrobial activity.

## 2.6 Phytochemical Screening

Phytochemicals are chemical compounds that occur naturally in plants. Some are responsible for color and other organoleptic properties. There may be as many as 4,000 different phytochemicals. It is well known that plants produce these chemicals to protect themselves, but recent researches demonstrate that they can also protect human against diseases.

Kumar and Seshadri (1976); Mitra and Joshi (1983) suggested that the *Pterocarpus marsupium* have showed the presence of bioactive compounds such as terpenoids, flavonoids, lupenol, neta sitosterol, epicatechin, flavonoids and phenols.

The ethyl acetate leaf extract of *Pterocarpus indicus* showed a mixture of paniculatadiol and loliolide and water-soluble polyphenols and tannins. The leaves of *Pterocarpus marsupium* reported the presence of terpenoids, flavonoids, flavanols, glycosides and phenols (Wang *et al.*, 1997 and Ramya, 2008).

Tchamadeu *et al.,* (2010) studied that the phytochemicals present in the extracts of *Pterocarpus indicus* revealed the presence of various chemical substances such as

tannins, terpenoids, quinines, proteins, alkaloids, flavonoids, carbohydrate, steroids, anthocyanin, phenols and stigma stool. The stem and barks of *Pterocarpus soyauxii* reported the presence of bioactive compounds such as tannins, flavonoids, terpenoids, steroids and phenols.

Gairola *et al.,* (2010) reported that the *Pterocarpus marsupium* contains pterostilbene (45%), alkaloids (0.4%), tannins (5%) and protein. Author demonstrated that the *Pterocarpus* belongs to the family Fabaceae contains various phytocompounds such as pterosupin, marsupsin, epicatechin, pterostilbene and flavonoids are used for medicinal purpose.

Badkhane *et al.,* (2010) and Tiwari *et al.,* (2015) reported that the *Pterocarpus marsupium* contains polyphenolic compounds, carsupin, marsupinol, pentosan, catechin, kinotannic acid, liquiritigenin, pterostilbene and epicatechin.

Hartati *et al.,* (2016) and Ouedraogo *et al.,* (2017) reported that the methanol, ethyl acetate and butanol extracts of *Pterocarpus erinaceus* roots showed the antioxidant activity for DPPH due to the presence of phytochemicals such as flavanol - glycoside [(2R)-7-hydroxy-3-(3, 4, 5- trihydroxy-6-(hydroxymethyl) tetrahydro-2Hpyran- 2-yloxy)-2-(3,4,5-trihydroxy phenyl) chroman-4-one] or ptevon-3-*D*- glycoside. Mohammed *et al.,* (2020) reported that the aqueous, ethanol and methanol of bark of *Pterocarpus marsupium* revealed the presence of tannins, terpenoids, steroids, alkaloids and phenols.

## 2.7 Antioxidant Activity of Synthesized Silver Nanoparticles

Banerjee and Narendhirakannan (2011) suggested that the biosynthesis of silver nanoparticles using a *Syzygium cumini* seed extract as reducing agent. UV visible spectroscopy was used for the quantification of silver nanoparticles synthesis.

Synthesized AgNPs characterized by using the SEM, EDAX, XRD and FTIR. The *in-vitro* antioxidant properties of the biosynthesized AgNPs were found higher antioxidant capacity compared to the seed extract thus have higher antioxidant potential radical scavenger against deleterious damages caused by the free radical.

Mittal *et al.,* (2012) the biosynthesis of stable and mono stable mono dispersed silver nanoparticles using *Rhododendron dauricum* flower extract. AgNPs were characterized in terms of synthesis, dynamic light scattering, FTIR spectroscopy and TEM, the specific characteristics and loss of organic content (1.81mg) of the synthesized nanoparticles was measured by DSC and thermo gravimeter analysis (TGA). The antioxidant activity of AgNPs imported by plant phenolic component was evaluated using DPPH assay and found to be comparable to standard TROLOX.

The plant of *Citrullus colocynthis* seed showed scavenging activity of free radical so it can be antioxidant agent. The silver nanoparticle synthesized from the marine algae *Ecklonia* showed antioxidant activity determined by DPPH scavenging assay (Benariba *et al.,* 2013 and Venkatesan *et al.,* 2016).

Demirbas *et al.,* (2016) demonstrated that the silver nanoparticles prepared using extract of red cabbage (*Brassica oleracea*) proposed that synthesized silver nanoparticles reduce silver ions, so AgNPs may promote superoxide radicals which could showed antioxidant activity of red cabbage.

The synthesized silver nanoparticles using *Costus afer* showed greater antioxidant activity than the leaf extracts. The synthesized silver nanoparticles from the aqueous leaf extract of *Elephantopus scaber* had strong scavenge DPPH free radicals (85.90%) and also silver nanoparticles by using green tea, garlic and turmeric extracts showed antioxidant activity (Elemike *et al.*, 2017 and Selvan *et al.,* 2018).

The antioxidant activity for thirty plant extracts and synthesized silver nanoparticles prepared by plants and found that the scavenging activity using DPPH assay increased when the concentration of the extracts increases than silver nanoparticles. The phenolic compounds 4-N-methyl benzoic acid isolated from *Memecylon umbellatum* and silver nanoparticles synthesized by using compounds showed the potential scavenge DPPH radical effect 81.57% (Ahn *et al.*, 2019 and AlSalhi *et al.*, 2019).

Chinnasamy *et al.*, (2019) reported that the silver nanoparticle prepared by using extract of *Melia azedarach* exhibited higher antioxidant activity as compared with the plant extracts due to the presence of phenolic compounds such as terpenoids, flavonoids and tannins which allow nanoparticles to act as singlet oxygen, reducing agents and hydrogen donors.

Kup *et al.*, (2020) reported that the silver nanoparticles synthesized by using *Aesculus hippocastanum* were used three different method of scavenging activity DPPH, superoxide anion radical scavenging assay and reducing power results showed the total reducing power of AgNPs indicated more reducing activity than the plants extracts but for DPPH and superoxide anion radical assays lead to reduced antioxidant capacity of AgNPs in comparison of plant extracts.

## 2.8 Antidiabetic Activity of Synthesized Silver Nanoparticles

The silver nanoparticles synthesized from *Halymenia poryphyroides* showed *in vitro* antidiabetic efficiency with an increase in percentage alpha-amylase inhibitory activity. The green synthesis of silver nanoparticles with *Tephrosia tinctoria* showed antidiabetic activity. UV-visible spectrophotometer, XRD, SEM, TEM, EDAX and FTIR analysis were characterized in this study. UV-Visible spectrum showed absorption peak at 480nm. EDAX analyses recorded 3KeV of elemental signal of silver nanoparticles as

49.86% of Ag in AgNPs. The phenol and flavonoids are the major compounds which enhance the bioactivity of synthesized AgNPs. This study showed better result for antidiabetic activity by increasing the uptake of glucose and significant free radical scavenging activity (Manam and Murugesan, 2013 and Rajaram, *et al.*, 2015).

Koli (2015) reviewed biologically synthesized zinc oxide nanoparticle with *Chellocostus speciosus* plant leaf extract and examined its anti-diabetic and anti-microbial agents. UV-Visible, FTIR and XRD analysis were analyzed in this study. UV-Visible spectrum showed absorption peak at 580nm and XRD confirmed the crystalline nature of AgNPs. The research showed good results for better antidiabetic activity using 1NS1E cell line and anti-microbial activity against *E. coli* and *S. aureus.*

Bhattacharya and Chakraverty (2016) demonstrated that *Annona squamosa* was studied for pharmacological properties and morphological characterization. These plants are identified by phytoconstituents is presented by alkaloids, isomeric hydroxyl ketones, and bark extracts. This also helpful to study antibacterial, antidiabetic, antitumor, antimalarial and antigenotoxic potential.

Balan *et al.*, (2016) reported that the silver nanoparticles using *Ananas comosus* exhibited promising anti-diabetic activity in a dose dependent manner. At a concentration of 1 µg/ml showed 100% inhibition of alpha glucosidase was observed and useful for the treatment of non-insulin diabetes because it will slow down the release of glucose in the blood.

Malapermal *et al.*, (2017) studied about silver nanoparticles with green method of *Ocimum basilicum* and *Ocimum sanctum* leaf extract and its antidiabetic and antimicrobial performance. UV-visible, TEM, SEM, EDX, zeta potential and DLS were characterized in this study. UV-Visible spectrum showed absorption peak at 439nm for

*Ocimum basilicum* and 433nm for *Ocimum sanctum.* It showed inhibitory activity of 89.31± 5.32% for *Ocimum sanctum* and 79.74±9.51% for *Ocimum basilicum* by alpha-glucosidase enzyme activity. This study revealed that the combined or either *Ocimum basilicum* nor *Ocimum sanctum* will helpful for the treatment of complications in diabetic patients. The result for this study examined that it has excellent antidiabetic and antimicrobial activity.

Govindappa *et al.,* (2018) evaluated the development of an easy and eco-friendly method for the synthesis of AgNPs with the aqueous leaf extract of *Calophyllum tomentosum* and studied about the effects of anti-bacterial, antioxidant, anti-diabetic, anti-inflammatory and anti- tyrosinase activity. UV-visible, XRD, FTIR, SEM and EDX were analyzed in this study. UV-Visible spectrum observed the Plasmon resonance at 438nm. XRD confirmed FCC and EDX showed strong absorption property of AgNPs. SEM analyses confirmed the spherical and uniform shape of AgNPs. This showed strong antioxidant activities, inhibitory activity against *S. aureus* and *K. aerogenes*, anti-diabetic, anti-inflammatory, anti-tyrosinase activity. Andleeb *et al.*, (2020) successfully synthesized the nanoparticles using *Allium sativum* bulb extract and showed to have a significant antibacterial, antidiabetic, anticoagulant and antioxidant effects.

## 2.9 Anticancer Activity of Synthesized Silver Nanoparticles

The author suggested that the silver oxide nanoparticles showed antitumor properties in transplanted Pliss lymphosarcoma tumour models. The author reported that the silver nanoparticles have found to induce the apoptotic pathway through *in vitro* condition for the free oxygen radical generation which showed antiproliferative, antitumor and antiangiogenic effects. The author suggested that the silver nanoparticles showed antiproliferative effect on human glioblastoma cells. The author suggested that the silver nanoparticle 5 nm were more toxic than 20 and 50 nm against A549, MCF-7,

SGC-7901 and HePG2. The author reported the effects of colloidal silver on MCF-7 human breast cancer cells (Rutberg *et al.*, 2008; Gurunathan *et al.*, 2009; Asharani *et al.*, 2009; Liu *et al.*, 2010; Franco-Molina *et al.*, 2010).

The cytotoxicity of nano silver is the consequence of dynamic physicochemical interaction of silver particles with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups of DNAs. The silver nanoparticle disrupted normal cellular function, membrane integrity and induce various apoptotic signalling genes of mammalian cells leading to cell death (Sriram *et al.*, 2010 and Sanpui *et al.*, 2011).

Gurunathan *et al.*, (2013) suggested a green method for the synthesis of water-soluble AgNPs by treating silver ions with hot aqueous extract of the mycelia of *Ganoderma neo-japonicum*. As a result, treatment of (MDA-MB-231) human breast cancer cells with various concentrations of AgNPs (1-10 μg/mL) for 24 hours revealed that AgNPs could inhibit cell viability and induce membrane leakage in a dose-dependent manner.

The silver nanoparticles shown anticancer activity against breast carcinoma (MCF-7), colon carcinoma (HCT-116), intestinal carcinoma ($CaCo_2$) and liver carcinoma (Hep-G2). The author suggested that the chemotherapeutic efficacy against multi-drug resistant cancer cells. The silver nanoparticles (10-20 nm) induce greater cytotoxicity than the larger one 110 nm and showed cell viability and DNA damage in *in vitro* condition (Shawkey *et al.*, 2013; Jiang *et al.*, 2013; Wang *et al.*, 2014).

Manikandan *et al.*, (2015) studied about the synthesis of silver nanoparticles (AgNPs) with ethanolic extract of rose petals (*Rosa indica*) and this novel study showed potential antibacterial activity using selective human pathogenic microbes, anticancer

activity using human colon adeno carcinoma cancer cell line HCT 15 as well as anti-inflammatory activity.

Nandagopal *et al.,* (2014) studied about synthesized silver nanoparticles with *Terminalia chebula* seed extract and their antibacterial and anti-cancer activities. Characterization technique like UV- Visible, SEM, TEM and statistical analysis were carried out in this study. UV-Visible spectrum observed the plasmon resonance of absorption peak at 429nm. The plant was found to possess active chemical compound for the treatment of various bacterial infections and better cytotoxicity effect by MTT assay.

Kanipandian *et al.,* (2014) reported that, the synthesized AgNPs were characterized by UV-Visible spectra, TEM, SEM, XRD and FTIR. This study results were crystalline in nature and the morphological studies revealed the spherical shape of AgNPs with size ranging from 20 to 40nm. AgNPs showed a better effect on scavenging of free radicals in antioxidant activity. This study also found that the cytotoxicity activity exhibited a dose dependent effect against human lung cancer cells (A549) and normal cells (HBL-100) the inhibitory concentration was found to be 30ug/mL and 60ug/mL.

The synthesized silver nanoparticles from *Piper longum* fruit extract showed cytotoxic effect against MCF-7 breast cancer cell lines. The genotoxicity of AgNPs is supported by the generation of double-stranded DNA breaks along with chromosomal instability that drives the initiation of apoptotic and also implies that AgNPs can be mutually associated with a great many DNA-targeting anticancer drugs (Reddy *et al.*, 2014; Souza *et al.*, 2016 and Ahmed *et al.*, 2017).

Phyto-synthetic method of producing silver nanoparticles (Ag-NPs) with size controlled is an eco-friendly route. The biosynthesized nanoparticles showed good cytotoxic impact against MCF-7, A549 and Hep2 cells compared to normal cell lines.

They have used AO/EtBr staining for the observations of the cell death induced by Ag-NPs. The fabricated AgNPs from *Millettia pinnata* flower extract showed antibacterial, anti-cholinesterase and cytotoxic activities (Venugopal *et al.*, 2017 and Rajakumar *et al.*, 2017).

Alahmad *et al.*, (2021) suggested that the green synthesis of silver nanoparticles with a size< 100 nm for medical applications by using the silver nitrate solutions and by aqueous extract of *Hypericum perforatum* showed high cytotoxicity by inhibiting cell viability for Hep G2, Hela and A549 cells.

### 2.10 *In Silico* Antidiabetic Activity using Phytocompounds from Medicinal Plants

The molecular docking is also known as hierarchical method which is powerful tool on computing and screening of protein structure to identify new ligand and also used to predicts molecules that bind with targets such as enzymes and receptor proteins. The ligand should have high affinity of small molecules with proteins to study the binding site of compound for a given receptor and leverage based on a scoring entity. The pharmacophore modelling is very important for lowering the drug cost and to determine the new drug most likely bind to target protein (Kitchen *et al.*, 2004; Gilson and Zhou, 2007; Yang 2010).

The docking with natural compounds is carried out in an active site of alpha glucosidase to score and calculate binding affinity in library. Acarbose has high docking score was predicted by molecular docking. The molecular docking is the fast screening of natural compounds. Docking is a computation of ligand with protein interaction for molecular recognition and quickly screen databases for protein structures and protein-ligand complexes for structure-based drug design (Brindis *et al.*, 2011; Wang *et al.*, 2013).

The pharmacophore model can be used to perform and identify small molecules in 2D or 3D protein structure and functional perdiction under *in silico* studies and also identify protein in human by the inhibitor and protein-ligand interactions for drug development. The docking of ligands against known enzymes allows for better understanding of the nature of the interaction between the phytochemical compounds of the extracts and both alpha amylase and alpha glucosidase. The docking data reported that the compound docosanol, tetracosanol, rutin, actinodaphnine, quercetin, berberine and catechin from 47 natural compounds had binding towards alpha amylase and alpha glucosidase (Qing *et al.,* 2014; Jamila *et al.,* 2015; Jhong *et al.,* 2015).

The docking scores for acarbose yields better inhibition with binding free energy of about -8.2 to -11.9 kcal/mol and for capsicum provided the binding score -5.8 to -6.1 kcal. mol. The amino acids such as Tyr82, Asp340, Tyr155 and Asp206 in the pockets of amylase and glucosidase have been previously identified as the common amino acids stabilizing the interaction of the two enzymes with ligands of antidiabetic drugs (Thongnum and Chanthai 2018; Sanni *et al.,* 2019).

Ahmad *et al.,* (2021) evaluated that the root extract of *Aristolochia ringens* showed inhibitory activity on alpha amylase and alpha glucosidase in silico studies and also reported that the identified compounds trilobine (-10.0, -10.0 kcal/mol) and dianoside G (-12.4, -12.5 kcal/mol) had significant interaction with alpha amylase and alpha glucosidase respectively. Asiatic acid and magnoflorine also interacted with enzymes quercetin 3-o glucuronide showed better affinity towards alpha glucosidase.

### 2.11 *In Silico* Anticancer Activity using Phytocompounds from Medicinal Plants

Sahu *et al.,* (2012) showed that the stigmasterol from *Avicennia marina* plant against the VP28 envelop protein by molecular docking. Hamsa *et al.,* (2013) has done

the prediction on molecular interaction of phytochemicals compounds against NFkB p50/p65 using molecular docking which showed that the compounds bilobetin, ginkgetin and mesuaxanthone B exhibited the best binding reactions.

Suganya *et al.*, (2014) suggested that the docking studies showed the compounds Chrysin and Equol were found to have the highest activation energy of -11.0189 kcal/mol and -10.8354 kcal/mol exhibits the best binding interaction with the human Estrogen Receptor and further used for the development of new preventive drug against breast cancer.

Ravichandran and Sundararajan, (2017) reported that, based on the drug screening scores phytochemicals Epi Gallo Catechin Gallate has shown the best binding affinity towards the BRCA1 receptor and energy score is -7.9 kcal/mol whereas standard drug Doxorubicin hydrochloride energy score is -8.1 kcal/mol. So, phytochemicals can be considered as an inhibitor against breast cancer susceptibility gene for breast cancer treatment.

Suryani (2018) revealed that the docking results showed the formononetin compounds have an energy score -7.3 kcal/mol lower than 17-β-Estradiol (natural estrogen) is -6.4 kcal/mol, but higher than 3 alkyl-naphthalene estrogen because it has a free energy.

Praveena *et al.*, (2019) studied that the compounds Corynan-17-ol, 18,19-dideyhdro-10-methoxy was selected as an efficient lead molecule against breast cancer protein ErBb2 target-based on the lowest binding energy value of -5.9 kcal/mol. This showed that the fruit of *Morinda tinctoria* used for identification of lead molecules against breast cancer.

Abdulrahman *et al., (*2020) reported that the molecular docking studies showed that ligand 15 and 18 had the highest score of -7.3 and -7.4 kcal/mol with thyroid hormone receptor (TRβ1) than the standard anti-breast cancer drug gefitinib (-5.3 kcal/mol).

## 2.12 *Pterocarpus indicus* and its applications

The methanol extract of stem bark of *Pterocarpus marsupium* have hepatoprotective activity. Toxic effect of CCl4 restoration of levels of protein, enzymes and serum bilirubin compared to normal drug silymarin- treated group. In human peripheral blood, *Pterocarpus marsupium* decrease, prostaglandin $E_2$ levels the inflammatory mediator cyclooxygenao -224. The root extract of *Pterocarpus indicus* is commonly known as rose wood are used to treat mouth ulcers, timber, fever, heavy menstruation and syphilitic sores (Rana *et al.*, 2000; Sanders 2002; Thomson, 2006).

The red, gum like resin from the bark of *Pterocarpus indicus* is used in folk remedies for tumours and its leaves used to cure mouth cancer and significantly inhibited the growth of Ehrlich as cites carcinoma cell in mice. The antidiabetic properties of ethanolic extract from stem bark of *Pterocarpus indicus* reduced blood glucose level dose was 200mg/kg (Orwa, 2009 and Malvi *et al.*, 2011).

Mohana *et al.*, (2012) suggested that the ethanolic extract of stem bark of *Pterocarpus indicus*, it lowers the glucose efficacy in both normal and alloxan induced diabetic rats. In both experimental animal models, the extract at the doses exhibited hypoglycaemic activity. The activity was compared to effective standard gilbenclamide-anti-diabetic agent.

# 3. Materials and Methods

## 3.1. Plant Collection and Authentication

The *Pterocarpus indicus* barks and leaves were separately collected from Senthankudi Village, Pudukkottai District. The barks and leaves were washed with normal water thoroughly for several times followed by distilled water to remove impurities. The shade dried bark and leaves were powdered using mechanical grinder. The plant was identified as *Pterocarpus indicus* (Vengai – Tamil Name) authenticated and confirmed by Dr. S. Soosairaj, Assistant Professor, Department of Botany, St. Joseph College, Tiruchirappalli, Tamil Nadu. The voucher specimen number is 2952.

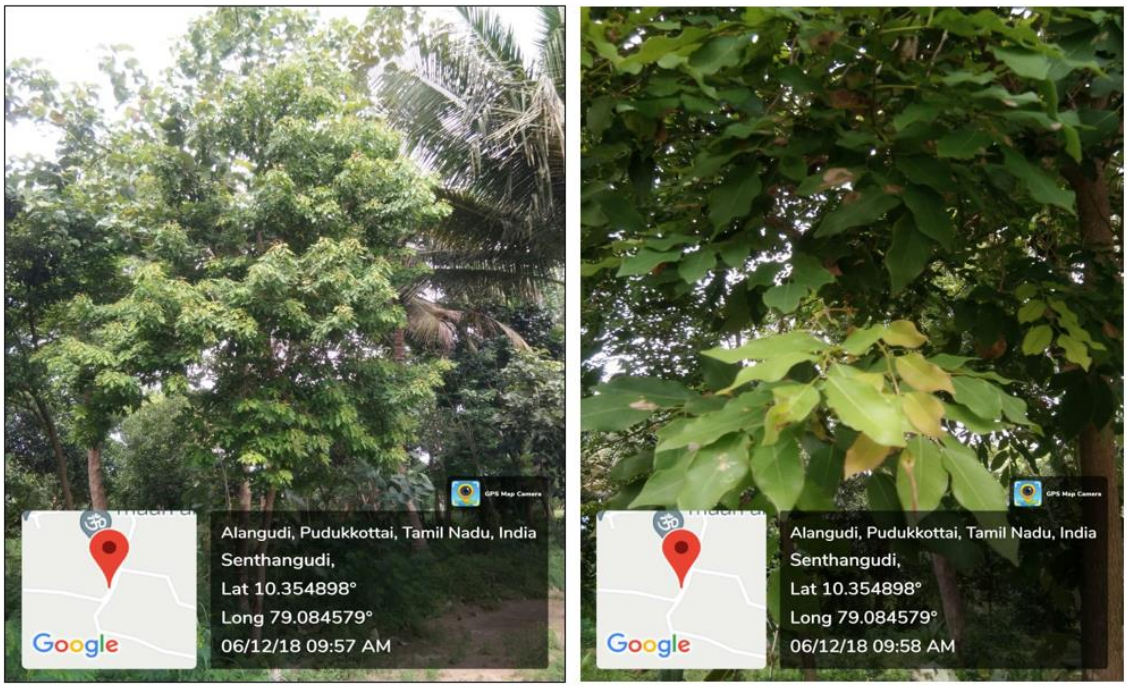



**Figure 1: *Pterocarpus indicus* Willd.**

### 3.2. Ethanol Extract of Plant Preparation

The measured amount of each powdered samples barks and leaves of *Pterocarpus indicus* were taken in a dry beaker and ethanol was added to it in a very few amounts. Now it becomes solution which was shifted in another clean beaker provided with a glass rod and watch glass. The change of colour from light green to dark green for leaves and for bark dark brown can be observed after boiling the solution for about 20 to 30 minutes. Then it was allowed to cool for 1 hour at normal room temperature. The filtration of the obtained extract was done using the whatman no.41 filter paper in another clean beaker. The obtained stock solution was taken in a brown bottle which must be kept in a cold condition for further analysis (Rathee *et al.*, 2015).

### 3.3. Phytochemical Screening of Ethanolic Extract of Barks and Leaves of ***Pterocarpus indicus*** (Harborne, 1998; Obadoni *et al.*, 2002 and Krishnaiah *et al.*, 2009)

**Test for Tannins:** 2ml of extract and 2ml of distilled water added in a test tube and filtered. Few drops of 0.1% ferric chloride were added and then filtered. Green precipitate indicates presence of tannins.

**Test for Philobatannins:** 2ml of extract and 2ml of 1% HCL was boiled to get red precipitate as the positive for the presence of philobatannins.

**Test for Saponins:** 5ml of extract and 5ml of water was added in a test tube. After adding the solution was shaken vigorously for the stable persistent froth. After shaking 3 drops of olive oil were added and shake to observe the formation of an emulsion.

**Test for Flavonoids:** 10% of lead acetate solution was added to 1ml of the extract. Change of yellow colour indicates the presence of flavonoids.

**Test for Steroids:** 2ml of the extract, 2ml of chloroform, 2 drops of sulphuric acid was added. Reddish brown ring indicates the presence of steroids.

**Test for Terpenoids:** 2ml of extract and 2ml of chloroform were added. Then two drops of con. sulphuric acid is added. To get reddish brown ring which indicates the presence.

**Test for Leucoanthocyanin:** 1ml of the extract, 1ml of isoamyl alcohol were added to the observation of organic layer into red as a positive and the presence of leuco anthocyanin.

**Test for Cardiac Glycosides:** 2ml of extract, and add 2ml of glacial acetic acid, and 1ml of ferric chloride and 1 drop of concentrated sulphuric acid was added. Appearance of violet or brown ring indicates the presence of cardiac glycosides.

**Test for Anthocyanins:** 2ml of extract, 1ml of Ammonia solution were added, pinkish red to bluish violet is obtained shows the presence of anthocyanin.

**Test for Anthraquinone:** 2ml of the extract, 2ml of ammonia solution and 1ml of benzene were added. Appearance of pink, violet or red colour. Presence of anthraquinone.

**Test for Proteins:** 2ml of extract, then 1ml of concentrated sulphuric acid were added and boiled. Appearance of white precipitate is obtained. Presence of protein.

**Test for Glycosides:** 2ml of extract, 2ml of chloroform and 2ml of acetic was added. Then the colour change from the violet to blue indicates the presence of glycosides.

**Test for Alkaloids:** 2ml of extract and add few drops of Hager's reagent were added and vigorously shaked for few minutes and gently to extract the alkaloids base. Presence of alkaloids.

**Test for Xanthoproteins:** 1ml of extract and 4 drops of ferric chloride were added. Appearance of blue-black colour. Presence of xanthoproteins.

**Test for Emodin:** 2ml of extract, 2ml of ammonium hydroxide and 3ml of benzene were added. Appearance of red is obtained. Presence of Emodin.

**Test for Carbohydrates:** 2ml of the extract, 2ml of distilled water, 2 drops of aqueous α-napthol and 2ml of concentrated sulphuric was added. Reddish violet ring is obtained. Presence of carbohydrates.

### 3.4. Quantitative Analysis of Ethanolic Extract of Barks and Leaves of *Pterocarpus indicus* (Harborne, 1973; Godghate *et al.*, 2012 and George, 2017)

**Test for Flavonoids:** To take the plant sample in 3g was extracted with the 10ml of distilled water and then filtered by using filter paper. To take clean test tube and then add 5ml of the filtrate and then mixed with 2ml of 0.1 ml ferric chloride in 0.1N Hydrochloric acid and 0.008M potassium ferrocyanide. And after 10 minutes the absorbance was measured by 300nm.

**Test for Saponins:** About 20ml of the condensed ethanol was prepared, and this concentrate were transferred to 250ml separating funnel and then add 10ml of dimethyl ether and vigorously shaking. And the ether layer is discarded, then aqueous layer is used. To add 10ml of n-butanol and 10ml of 5% of NaCl and then heated and placed in water bath. Then after the evaporation the sample was dried and weighed.

**Test for Alkaloids:** About 5g of sample are taken and boiled with the 10ml of 10% acetic acid in ethanol to stand for 2hours. And add ammonium hydroxide to take the whole solution was allowed to settle and precipitate were collected with dilute ammonium hydroxide and then filter, dried and weighed.

**Test for Phenol:** To take a small amount of the sample was boiled with dimethyl ether. And the added by 5ml of the extract,10ml of the distilled water and 2ml of ammonium hydroxide and 5ml oh concentrated amyl alcohol. After 30minutes the reaction takes place in the development of colour is obtained and this is measured for 505nm.

**Test for Terpenoids:** About 10ml of sample were taken by soaked in alcohol for 24hours. Then the filtrate is extracted by petroleum ether for 2hours and then transferred into crucible, dried and weighed.

### 3.5. Optimization and Synthesis of Silver Nanoparticles

Barks and leaves of *Pterocarpus indicus* were used as a stock solution. The amount of perfect silver nitrate is taken in the 50ml standard flask and the solution is liquefied with the deionised water. To close the flask, aluminium foil is used to prevent photochemical reactions in advance. Without any contamination the stock solution in different concentration of barks and leaves extract (25µl, 50µl, 75µl and 100µl) are mixed with 1mm of silver nitrate solution separately. Then the silver nanoparticles are formed by the solution was kept at room temperature. The formation of silver nanoparticles in a transparent manner is confirmed by colour change of colourless to red-brown. This occurs without any agglomeration and very stable in nature (Ahmed *et al.*, 2016).

### 3.6. Characterisation Techniques (Subbaiya *et al.*, 2014; Krishnaraj *et al.*, 2010)

#### 3.6.1 UV-Visible Spectroscopy

UV-Visible spectroscopy is used to measures then the optical properties (shape, concentration, size, refractive index and agglomeration state) by the nanoparticle's eradication (absorption + scatter) of the light passes through the sample. Hence these

characterized studied by the UV-Visible spectroscopy. When light passes through a sample its extinction was measured by the UV-Visible spectroscopy.

The size, shape, concentration, agglomeration state, and refractive index near the nano article surface has unique optical properties for identifying, characterizing, studying nanomaterial made UV-visible as a valuable tool. UV-vis spectroscopy is the most important technique and the simplest way to confirm the formation of nanoparticles. Synthesized silver nanoparticles were confirmed by sampling the aqueous component of different time intervals and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 300-700nm with distilled water as a reference (UV-VIS Spectrometer2202).

### 3.6.2 FTIR Analysis

FTIR is abbreviated as Fourier Transform Infrared Spectroscopy. This technique is otherwise called as FTIR Analysis or FTIR Spectroscopy. In this method observed by the materials like organic, inorganic and polymeric materials by using the chemical properties and infrared light is scanned by the following test sample. Then the range of Fourier Transform Spectrometer records the infrared spectra is 400-450cm-1.

IR absorption of the functional group may vary over a wide range is due to the complex interaction of atoms within the molecule. Multiple functional groups may be absorbed at a particular frequency and it gives rise to several characteristic absorptions. FTIR analysis was performed to classify the biomolecules which were responsible for reduction of the metals and for the stabilization of nanoparticles. The functional group responsible for the silver nanoparticles also analyzed using FTIR RX1-Perkin Elmer.

### 3.6.3 SEM Analysis

SEM is the light- based imaging that uses an electron beam to image a nanoparticle size. Then its analysis to measure the size distribution and morphology of the nanoparticles. SEM were performed to known the size and shape of bio-reduced silver nanoparticles. Purified silver nanoparticles were sonicated for 15 min to make it uniform distribution and a drop of this solution was loaded on carbon-coated copper grids and solvent was allowed to evaporate under infrared light for 30 min. Sem experiments were performed on Icon Analytical, FEI Quanta 220.

### 3.6.4 XRD Analysis

XRD is otherwise called as X-ray diffraction. The nanomaterial structure has been probed by using the XRD method. And the XRD method is study of the nanomaterials. (Range 1-100nm) position of values of product is find by using these methods (crystallinity or amorphous nature). The interplanar d-spacing and the relative intensities ($I/I_o$) of the strongest peak of XRD pattern were characterized under the Hana watt system. This technique is used to find the position of values of product crystallinity or amorphic nature.

The fingerprint region of relative intensity is found using the data with respect to d-spacing values. X-ray diffraction (XRD) analysis was conducted by XPERT_PRO using monochromatic *cu ka* radiation (=1.5406 $A^0$) operator at 40Kv and 30mA at a 2theta angle pattern. The scanning as done in the region of $20^0$-$80^0$. The images obtained were compared with the joint committee on powered diffraction standards (JCPDS) library to account for the crystalline structure.

### 3.6.5 ZETA Potentiometer

The zeta potential was measured by using Zeta Sizer (Malvern Instruments) having zeta cells, polycarbonate cell with gold-plated electrodes and using water as medium for sample preparation. Zeta potential determines the surface potential of silver nanoparticles and it is essential for the characterization of stability of nanoparticles. The stability of nanoparticles is measured when the values of zeta potential ranged from higher than +30 mV to lower than -30 Mv.

## 3.7 Antioxidant Activity of Synthesized Silver Nanoparticles using Barks and Leaves of *Pterocarpus indicus*

### 3.7.1 DPPH Assay Method

The antioxidant activity of synthesized silver nanoparticles was based on the procedure of (Braca *et al.*, 2002). The nanoparticles were taken in a dry test tube (20-100µg/ml) are made up into 1ml of ethanol and mixed well. Take two test tubes and transfer the solution are mixed well and poured into the two test tubes with the different concentrations (20µg/100µg/ml). In dark area the freshly prepared DPPH solution was kept at 4ºC. In all tubes 500µl of DPPH solution was added. In spectrophotometrically, for 5 min absorbance was measured at 517nm and mixture was kept as constant. Ethanol was used to set the absorbance at zero.

A blank sample was also prepared which contains 1ml of ethanol and 500µl of DPPH solution for the preparation. In triplicate all the determinations were performed. According to the equation it is calculated as percentage of inhibition, the radical scavenging activities of the tested samples were

**DPPH activity (% inhibition) = [(A – B) / A] x 100**

A and B represents the absorbance value for the test and blank sample. The percent inhibition versus concentration curve and 50% inhibition was determined in a sample where the graph is plotted.

### 3.7.2 Reducing Power Method

The synthesized silver nanoparticles using barks and leaves of *Pterocarpus indicus* were taken in different concentrations in phosphate buffer (pH 6.6/ 0.2mol/L), and incubated with potassium ferricyanide (1g/100ml of water) at the 50˚ C for 20 minutes, then the reaction was terminated by adding TCA solution (10g / 100ml water) and centrifuged at 300rpm for 10 minutes and the ferric chloride was mixed (0.1g / 100ml of water), at 700nm the absorbance was measured. The increased in the absorbance of reaction mixture indicates increases reducing power (Kim *et al.*, 2005).

## 3.8. Antidiabetic Activity of Synthesized Silver Nanoparticles using Barks and Leaves of *Pterocarpus indicus*

### 3.8.1 Alpha Amylase Inhibition Assay

The alpha-amylase inhibitory assay brings out with the procedure of Tchimene *et al.* (2016). Into a test tube (20- 100µg/ml) different concentration of synthesized silver nanoparticles each was taken and made up to 1ml with ethanol and mixes it. Then a total of 250µL of this solution was transferred into a new tube with different concentrations (20- 100µg/ml) and 250µL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was filled. This solution or result was pre-incubated at 25ºC for 10 minutes. 250µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at regular time intervals and then further incubated at for 25ºC for 10minutes. Finally, 500µL of dinitro salicylic acid (DNS) reagent were added. The test tubes were incubated in boiling water for 5minutes and cooled in room temperature. The reaction mixture was diluted by adding 5mL distilled water and the absorbance was

measured at 540nm using spectrophotometer. The control of process was also prepared as same procedure as 250 µl of distilled water. The α-amylase inhibitory activity was calculated in terms of percentage inhibition as follows;

**%Inhibition = [(Abs control – Abs aqueous extract) / Abs control] x 100.**

A and B represents the absorbance value for the test and blank sample. The percent inhibition versus concentration curve and 50% inhibition was determined in a sample graph is plotted.

### 3.8.2 Alpha Glucosidase Inhibition Assay

The activity of synthesized silver nanoparticles each bark and leaves on α-glucosidase was determined by α-glucosidase from *Saccharomyces cerevisiae* (Ramkumar *et al.,* 2010). Into a test tube (20- 100µg/ml) different concentrations of synthesized silver nanoparticles each were taken and made up to 1ml with ethanol and mix it. Then, a total of 250µL of this solution was transferred into a new tube with different concentrations (20- 100µg/ml). The P-nitro phenyl glucopyranoside (pNPG) was prepared in 20mM phosphate buffer as a substrate solution and pH 6.9. 100µL of α-glucosidase (1.0 U/mL) was pre-incubated with 50µL of the different concentrations (20-100µg/ml) of the synthesized silver nanoparticles each for 10min. After that 50µL of 3.0mM (p-NPG) substrate was dissolved in 20mM phosphate buffer (pH 6.9) were added to start the reaction. At 37ºC for 20min and made up with 2mL of 0.1M $Na_2CO_3$ this reaction mixture was incubated. The α-glucosidase activity was determined by measuring the yellow-coloured p-nitro phenol released from pNPG at 405nm. The α-glucosidase inhibitory activity was calculated by percentage of inhibition as follows;

**%Inhibition = [(Abs control – Abs aqueous) / Abs control] x 100.**

A and B represents the absorbance value for the test and blank sample respectively. The percent inhibition versus concentration curve and 50% inhibition was determined in a sample. where the graph is plotted.

### 3.9. *In vitro* Anti-Cancer Studies of Synthesized Silver Nanoparticles using Barks and Leaves of *Pterocarpus indicus*

**Principle**

MTT (3,4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) assay is fully depends on the ability of a mitochondrial dehydrogenase enzymes of viable cell to cut the tetrazolium rings of the pale yellow MTT and dark blue coloured formazan crystals formed which is impermeable to cell membrane, that results in accumulation within the healthy cells which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created and the formed color can be analyzed using a multi-well plate reader.

**Procedure** (Manikandan *et al.*, 2012)

**Cell Culture:** MCF-7 (human breast carcinoma cells) cell line was cultivated in liquid medium (DMEM) with addition of 10% Fetal bovine serum and 100 µg/ml of penicillin.

**MTT Assay** (Gajendran *et al.*, 2014)

The synthesized silver nanoparticles of barks and leaves of *Pterocarpus indicus* each separately were tested for *invitro* cytotoxicity, using MCF-7 cells by 3,4,5 dimethyl thiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the MCF-7 cells were collected by trypsinization in 15 ml test tubes. Then, the cells were plated into 96-well tissue culture plate in DMEM medium containing 1% antibiotic solution and 10% FBS for 24-48 hours at 37 ºC. Then the wells were washed with sterile PBS and treated with synthesized silver nanoparticles barks and leave each in a serum free DMEM

medium. Each cell was incubated at 37º C in a humidified 5% carbon dioxide incubator for 24 hours and each sample were replicated three times.

After the incubation period, MTT (20 of 5 mg/ml) was added into each well and the cells incubated for another 2-4 hours until purple precipitates were clearly visible under an inverted microscope. Finally, the medium was aspirated off the wells and washed with 1XPBS (200 µL). And then DMSO (100 µL) was added and the plate was shaken for 5 min until to dissolve formazan crystals and each well separately were measured at 570nnm using a micro plate reader and the percentage of cell viability and IC 50 value was calculated using graph pad prism 6.0 software.

### 3.10. GC-MS Analysis of Bioactive Compounds from the Ethanolic Extract of Barks and Leaves of *Pterocarpus indicus*

The bioactive compounds investigation of ethanolic extract of barks and leaves each separately were performed on a GC-MS equipment (GC-MS QP2010 Plus, Shimadzu, Kyoto, Japan) system. The system was equipped with an auto injector (AOC-20i), head space sampler (AOC-20s), a mass selective detector with an ion source (220 °C) and an interface (260 °C). Rtx-5 MS capillary column having 30 mm X 0.25 mm of length X diameter and 0.25 µm of film thickness was used for MS analyses. The injector was set in the split injection mode having 250 °C of temperature. The ratio applied for split mode was 10.0. The starting temperature was adjusted to 80 °C (3 min), which afterwards increased to 280 °C with a ramp rate of 10 °C/min. Helium (>99.99 %) with 40.5 cm/s of linear velocity was employed as a carrier gas. The system was programmed with 16.3 ml/min of total flow rate and 1.21 ml/min of column flow. Components were recognized by their retention time (RT) and elucidation of mass spectra. The spectral fragmentation of unknown components was compared with the known and standard

components provided by the databases of WILEY8LIB (Sridharan *et al.*, 2011; Siddiq Ibraham *et al.*, 2009).

### 3.11. *In-Silico* Molecular Docking Analysis of Bioactive Compounds from Barks and Leaves of *Pterocarpus indicus* for Alpha Amylase and Alpha Glucosidase Inhibitory Activity

#### 3.11.1. Preparation of Protein Structure

The 3D structure of alpha amylase (PDB 1PP1) and alpha-glucosidase (PDB 2ZE0) were obtained from the Protein Data Bank (http://www.rcsb.org/pdb). Water molecules were removed from the protein 1PP1 and 2ZE0 prior to the docking calculation with the help of Protein Preparation Wizard icon of Maestro program by Schrodinger.

#### 3.11.2. Preparation of Ligand (Shelley *et al.*, 2007)

The structure of compounds Phthalic acid, butyl ester, ester with butyl glyc, Cytidine (CAS) Cyd , Bi-1,3,5-cycloheptatrien-1-yl (CAS), Phthalic acid, di-(1-hexen-5-yl) ester, 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon, Silane, [1-(5-hexenyl)-2methylenecyclopropy, Zingiberene, Cyclopentane, heneicosyl- (CAS) Heneicosane, 1,3-dioxolane,2-(phenylmethyl)-, Benzoldicarbonsaeure, dl-Citronellol, 9,12,15-Octadecatrienoic acid, methyl ester, 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol, 5,8,11,14-Icosatetraynoic Acid, Nonadecnoic Acid, 1, E-11, Z-13-Octadecatriene, Docosane, Hexadecanoic acid (CAS) Palmitic acid, 9-Octadecenoic acid (Z)- (CAS) Oleic acid, 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth, 14-Heptadecenal, 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl, 1,2,3-Propanetriol, diacetate (CAS) Diacetin, Propanoyl chloride, Methane, Thiobis-, n- Allyloxymethyl acrylamide, Dodecanoic acid (CAS) Lauric acid, 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy, Trans-2-Phenyl-1,3-

Dioxolane-4-M were selected based on the GCMS data and prepared as per the guidance of Maestro ligand preparation wizard.

### 3.11.3. Glide Molecular Docking Analysis (Friesner *et al.,* 2006)

To find out the accurate binding model for the active site of alpha amylase and alpha glucosidase enzymes, molecular docking analysis was determined using ligand fit of GLIDE software from Schrodinger (http://www.schrodinger.com). GLIDE molecular docking supports one ligand to interact with the structure of the target proteins for evaluation of the active site receptor grid and were created using the receptor grid generation option of Maestro. GLIDE module of the XP analyzed the specific ligand-protein interactions. The phytochemicals were docked with the 3D structure of the enzyme's alpha amylase and alpha glucosidase with the help of GLIDE. The docking scores were obtained.

## 3.12. *In Silico* Docking Analysis of Phytochemicals from the Barks and Leaves of *Pterocarpus indicus* for Human Estrogen Receptor

### 3.12.1. Protein Preparation

Three-dimensional protein structure of human estrogen receptor (PDB: 3ERT) was downloaded from RCSB site (www.rcsb.org). Structure was arranged with the help of Protein Preparation Wizard of Schrodinger-Maestro program.

### 3.12.2. Ligand Preparation

The structure of the Compounds as mentioned above for *in silico* study of antidiabetic activity were retrieved from PubChem databases and the standard Tamoxifen (TM) were selected based on their bioactive data. Ligands were prepared as per the guidelines of Maestro ligand preparation wizard.

### 3.12.3. Estrogen Receptor Grid Generation

GLIDE molecular docking supports one ligand to interact with the X-ray crystal structure of the target protein for evaluation of the active site receptor grid. Receptor grid dependent molecular docking helps the ligands to bind in many possible conformations. Docking grids for the protein structure 3ERT were created using the receptor grid generation option of Maestro.

### 3.12.4. Glide Molecular Docking

Molecular docking was performed by using GLIDE and its outputs represented as the docking scores. GLIDE module of the XP molecular docking studies using the selected ligand molecules were conducted using Maestro molecular docking. Each of the selected phytochemical compounds were docked into target protein molecules 3D structure of ERα (3ERT) with the help of the GLIDE. The final energy evaluation was done on the basis of docking score.

### 3.12.5. ADME Properties Studies

The pharmacokinetic properties were observed using SWISS ADME (http://www.swissadme.ch/) an online web server. pkCSM ADMET web server was used to determine the absorption, distribution, metabolism, excretion and toxicity of ligands (Han *et al.,* 2019).

## 3.13. Statistical Analysis

All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at $P < 0.05$ were considered to be significant.

# 4. Results

## 4.1 Phytochemical Screening of Bioactive Compounds from the Leaves and Barks of *Pterocarpus indicus*

The presence of various phytochemical compounds viz., tannin, phlobatannin, saponin, flavonoids, steroids, terpenoids, anthocyanin, anthraquinone, coumarin, phenol, cardiac glycoside, xanthoprotein, alkaloids, emodin and carbohydrates were analyzed in ethanolic extracts of leaves and barks of *Pterocarpus indicus* (Table 1).

**TABLE 1: Identification of Active Constituents from the Leaves and Barks of *P. indicus***

| S. No | Phytochemical Compounds | Observation | Barks | Leaves |
|---|---|---|---|---|
| 1 | Tannin | Brownish green | +++ | +++ |
| 2 | Phlobatannin | Red precipitate | +++ | + |
| 3 | Saponins | Blue color | ++ | +++ |
| 4 | Flavonoids | Yellow color | +++ | +++ |
| 5 | Steroids | Blue color | ++ | +++ |
| 6 | Terpenoids | Reddish brown | +++ | + |
| 7 | Cardiac glycosides | Brown ring | +++ | +++ |
| 8 | Leucoanthocyanin | Red layer | + | + |
| 9 | Anthocyanin | Bluish violet | ++ | + |
| 10 | Anthraquinone | Red | +++ | + |
| 11 | Coumarin | Yellow color | +++ | +++ |
| 12 | Protein | White precipitate | + | + |
| 13 | Glycosides | Green color | ++ | +++ |
| 14 | phenol | Blue Black | +++ | +++ |
| 15 | Xanthoprotein | Reddish orange precipitate | ++ | - |
| 16 | Alkaloids | Yellow color | +++ | +++ |
| 17 | Emodin | Red color | +++ | ++ |
| 18 | Carbohydrates | Reddish violet color | +++ | ++ |

**+++: strongly present, ++: moderately present, +: slightly present, -: absent**

## 4.2 Quantitative Estimation of Important Secondary Metabolites from the Leaves and Barks of *Pterocarpus indicus*

The ethanolic solvent extracts contained the maximum quantity of phytochemicals (as evident from qualitative screening) were selected for the quantification of secondary metabolites (Table 2). The phytochemicals with the highest quantity of active compounds were quantified by leaves showed flavonoids (0.036mg/g), tannin (0.083mg/g), saponin (0.051mg/g), alkaloids (0.057mg/g), phenols (0.024mg/g), terpenoids (0.028mg/g) respectively. The barks of *Pterocarpus indicus* showed tannin (0.053) followed by flavonoids (0.018mg/g), saponin (0.020mg/g), alkaloids (0.025mg/g), phenols (0.010mg/g), terpenoids (0.012mg/g) respectively.

**TABLE 2: Quantitative Determination of Important Secondary Metabolite from the Leaves and Bark of *Pterocarpus indicus***

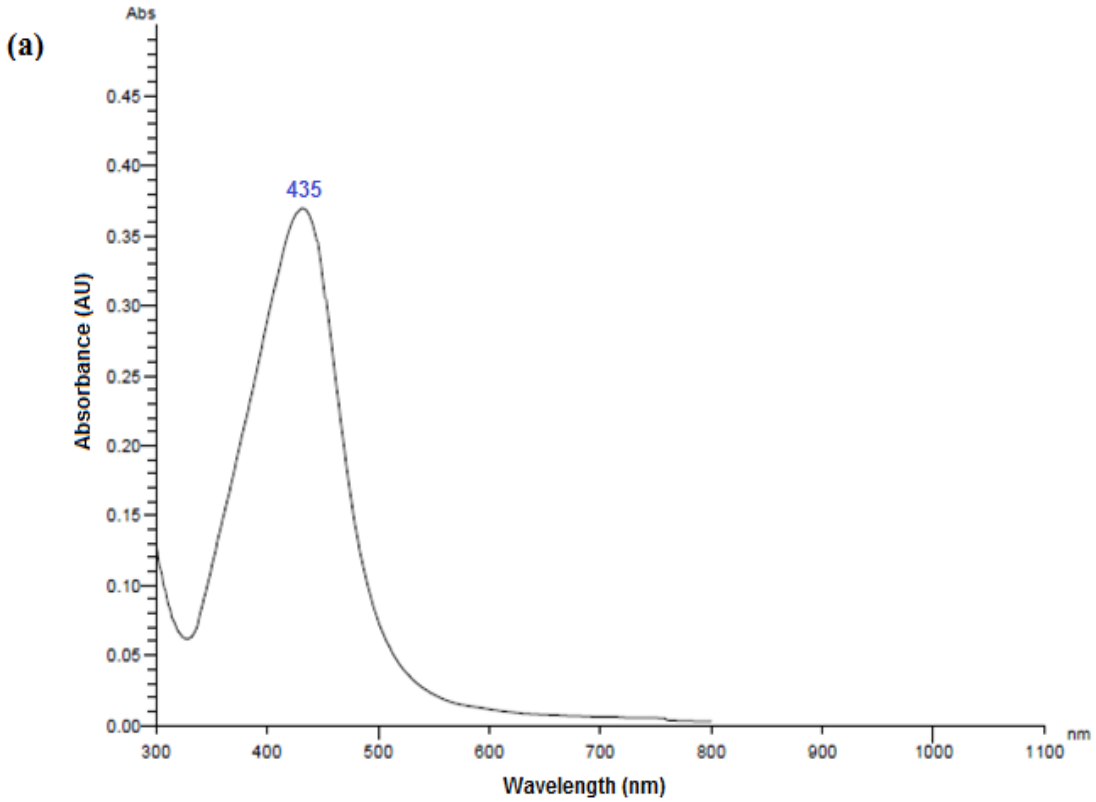
| S. No | Phytochemical Constituents | Bark (mg/g) | Leaves (mg/g) |
|---|---|---|---|
| 1. | Flavonoid | 0.018 | 0.036 |
| 2. | Tannin | 0.053 | 0.083 |
| 3. | Saponin | 0.020 | 0.051 |
| 4. | Alkaloid | 0.025 | 0.057 |
| 5. | Phenol | 0.010 | 0.024 |
| 6. | Terpenoid | 0.012 | 0.028 |

## 4.3 Synthesis and Characterization of Silver Nanoparticles using Leaves and Stem Bark of *Pterocarpus indicus*

### 4.3.1 Observation of Visual Color Change by UV-Vis Spectroscopy

The experiment was carried out by adding ethanol in leaves and barks extract of *Pterocarpus indicus* each separately add in to the glass vial which contains $AgNO_3$, the change in color from colorless to reddish brown for barks extract and for leaves the

gradual change in the colour of samples from light green to dark brown colour was observed which denotes the presence of silver nano particles. The presence of silver nanoparticles was viewed by using the UV-Vis spectral technique at room temperature. The UV visible absorption spectrum was noted at the range of 435 nm for the leaves-silver nanoparticles and at the range of 439 nm for the stem bark-silver nanoparticles (Figure 2).



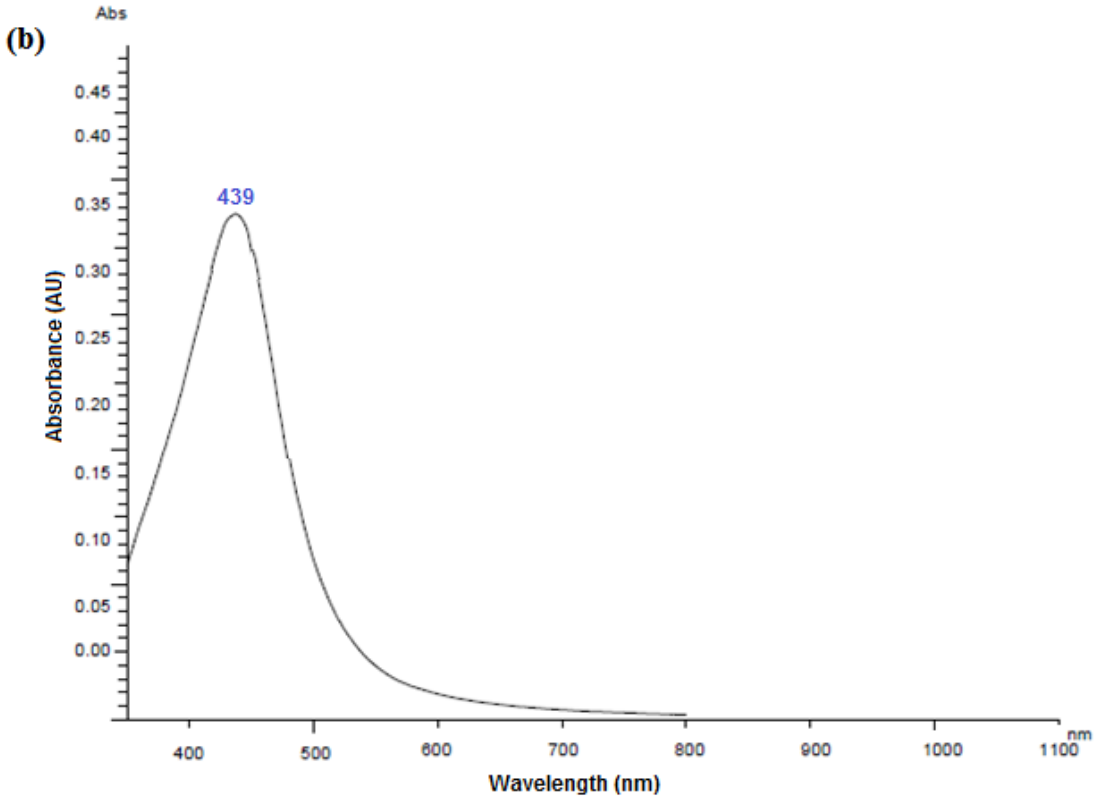


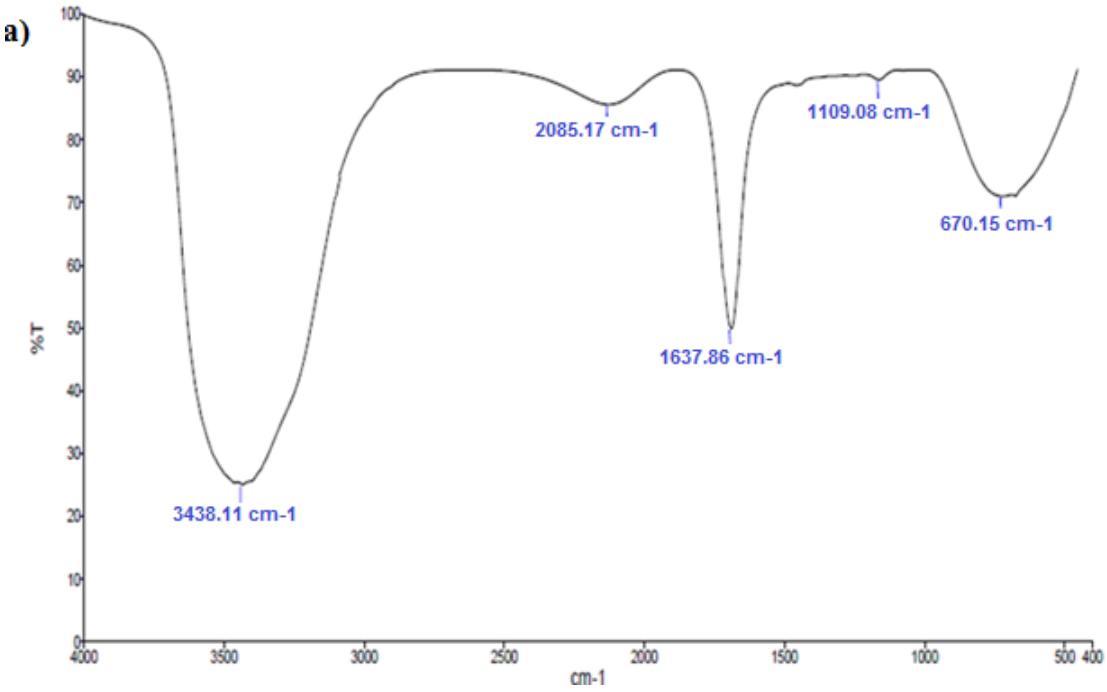

**FIGURE 2: UV-Vis Spectrum of Synthesized Silver Nanoparticles using *Pterocarpus indicus* (A) Leaves-AgNPs (B) Stem Bark-AgNPs**

### 4.3.2 Functional Group Determination using FT-IR Spectroscopy

The FT-IR spectrum of synthesized silver nanoparticles gives information about the functional group involved in the silver ions reduction (Figure 3). The following peaks are observed in the leaves-AgNPs, the medium band primary amine at 3438.11 $cm^{-1}$ corresponds to N-H stretching vibrations. The strong band Isothiocyanate at 2085.17 $cm^{-1}$ corresponds to N=C=S stretching vibrations. The medium band Alkene at 1637.86 $cm^{-1}$ corresponds to C=C stretching vibrations, Amine at 1109.08 $cm^{-1}$ corresponds to C-N stretching vibrations. The Strong band Halo compound at 670.15 $cm^{-1}$ corresponds to C-Br stretching vibrations (Figure 3a). For the stem bark-AgNPs, the strong band primary amine at 3441.16 $cm^{-1}$ corresponds to 3441.16 $cm^{-1}$ corresponds to broad O-H

stretching alcohol. The Strong band Isothiocyanate at 2077.79 $cm^{-1}$ corresponds to N=C=S stretching vibrations.

The Medium band Alkene at 1634.81 $cm^{-1}$ corresponds to C=C stretching conjugated alkene and amine at 1405.54 $cm^{-1}$ corresponds to O-H bending alcohol. The Strong band Alcohol at 1114.45 $cm^{-1}$ corresponds to C=O secondary alcohol, Anhydride at 1030.7 $cm^{-1}$ corresponds to CO-O-CO anhydride and Halo compound at 662.74 $cm^{-1}$ corresponds to C-Br stretching vibrations (Figure 3b). Analysis of these spectra strongly suggested the presence of flavonoids and phenols, which were mainly responsible for the formation of silver nanoparticles by reducing silver nitrate.



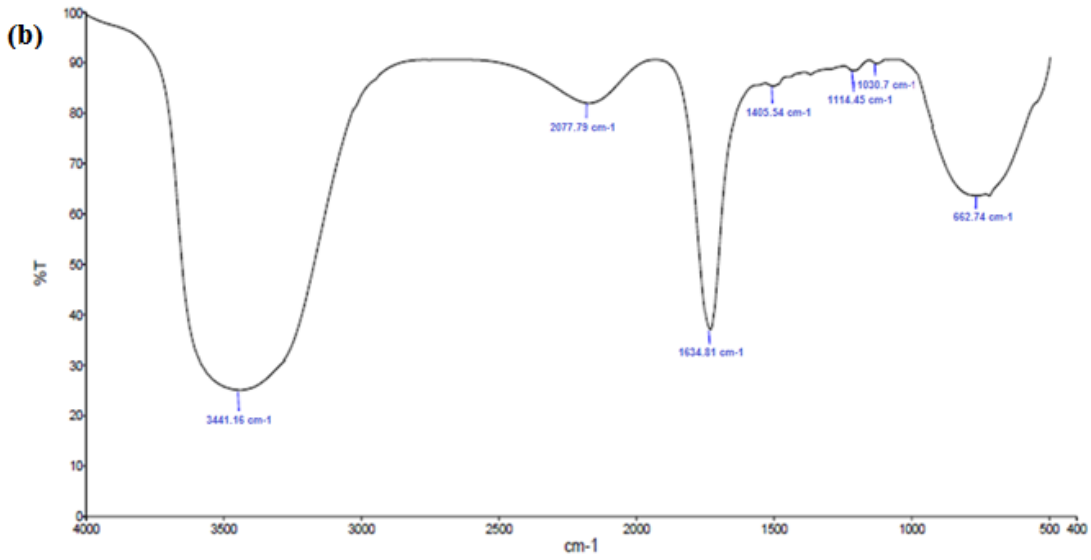



**FIGURE 3: FTIR Spectrum of Synthesized Silver Nanoparticles using *Pterocarpus indicus* (a) Leaves-AgNPs (b) Stem Bark-AgNPs**

### 4.3.3 X-ray Diffraction (XRD)

In X-ray crystallography the crystalline nature of silver nanoparticles was confirmed using the leaves and stem bark of *Pterocarpus indicus* (Table 3 and 4). The synthesized silver nanoparticles pattern performed by XRD was represented in (Figure 4). The diffraction peaks were obtained by leaves-AgNPs is observed at 38.4024, 46.3896, 65.1721 and 78.568 in the 2Ɵ range (Figure 4a). The obtained XRD pattern for silver nanoparticles synthesized using *Pterocarpus indicus* bark extract showed the characteristic peaks 38.7965, 44.7206, 65.0686 and 78.2134 in the 2Ɵ range (Figure 4b) respectively. The pattern (111), (200), (220) and (311) observed was face cantered cubic structure for silver according to (JCPDS, File No. 04-0783). The peaks obtained from the graph was unassigned may be due to extract contains some phytochemicals which may be capping the nanoparticles surface.

**TABLE 3: Synthesized Silver Nanoparticles using *Pterocarpus indicus* Leaf Extract were Analyzed by XRD Patterns**

| S. No | 2 Theta | FWHM Degree | Inter Planar Spacing, d-spacing [Aº] |
|---|---|---|---|
| 1 | 38.4024 | 0.5904 | 2.34408 |
| 2 | 46.3896 | 0.2460 | 1.95740 |
| 3 | 65.1721 | 0.1476 | 1.43146 |
| 4 | 78.5638 | 0.1200 | 1.21966 |

**TABLE 4: Synthesized Silver Nanoparticles using *Pterocarpus indicus* Bark Extract were Analyzed by XRD Patterns**

| S. No | 2 Theta | FWHM Degree | Inter Planar Spacing, d-spacing [Aº] |
|---|---|---|---|
| 1 | 38.7965 | 0.0276 | 0.2337 |
| 2 | 44.7206 | 0.0182 | 0.2038 |
| 3 | 65.0686 | 0.0322 | 0.1442 |
| 4 | 78.2134 | 0.0239 | 0.1224 |

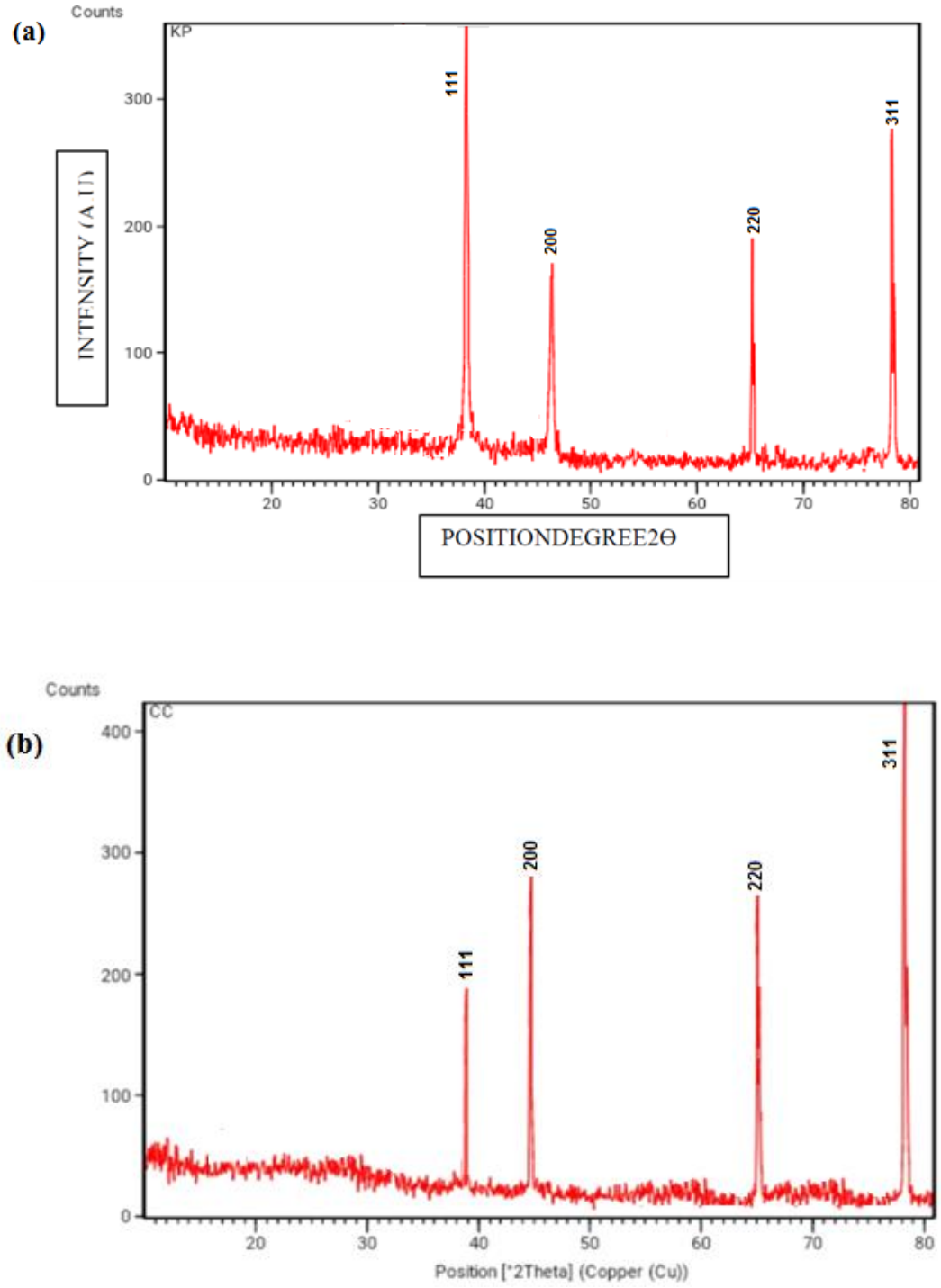
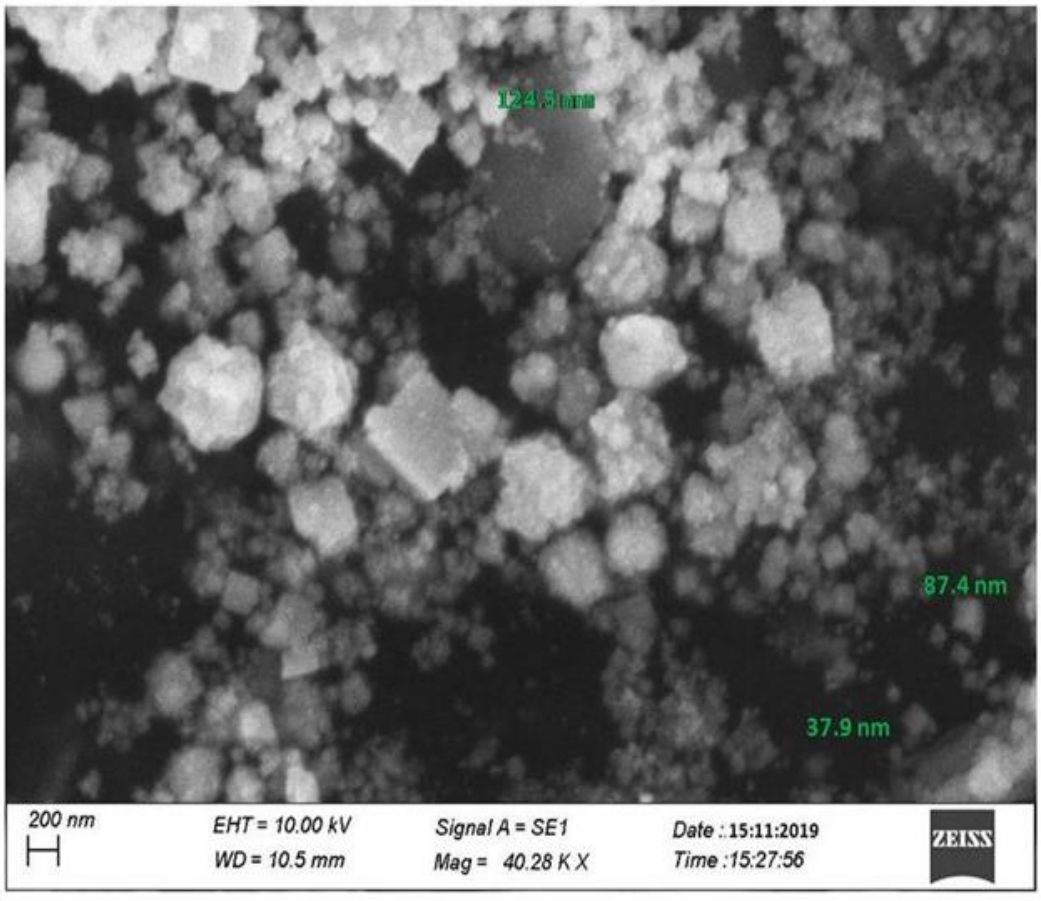



**FIGURE 4: XRD Analysis of Synthesized Silver Nanoparticles using *Pterocarpus indicus* (a) Leaves-AgNPs (B) Stem Bark-AgNPs**

### 4.3.4 SEM Analysis

SEM image reveals most of the synthesized silver nanoparticle was spherical in shape and well dispersed (Figure 5). From the SEM image it was concluded that synthesized silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* were almost uniform in shape and size. SEM images of the synthesized silver nanoparticles lie between 37.9-124.5 nm region in case of leaves-AgNPs (Figure 5a) and 98.70-126 nm in case of stem bark-AgNPs sample (Figure 5b), the average size of the nanoparticles is ~ 200 nm, whereas the shapes were spherical and cubic.

(a)



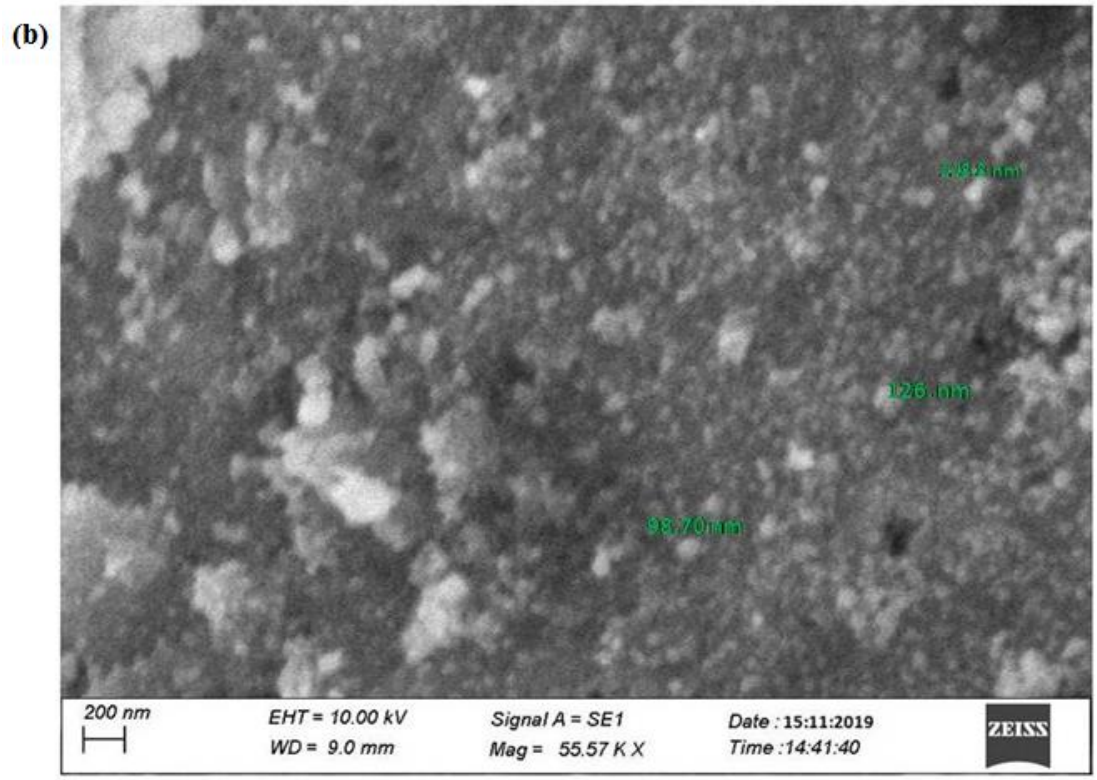



**FIGURE 5: SEM Images of Synthesized Silver Nanoparticles using *Pterocarpus indicus* (a) Leaves-AgNPs (b) Stem Bark-AgNPs**

### 4.3.5 Particle Size Distribution and Zeta Potential Studies

Particle size distribution analyzer is performed to analysis the particle size distribution, average size of the nanoparticles and polydispersity index (PDI) of the synthesized silver nanoparticles. Dynamic light scattering results also confirmed an average size of the biosynthesized silver nanoparticles was in nanometre range. The intensity weighed particle size distribution histograms are obtained from the leaves-AgNPs exhibited polydisperse mixture with the size ranging of 1842 nm with poly dispersity index of 0.247 (Figure 6a) and for the stem bark-AgNPs exhibited polydisperse mixture with the size ranging of 385 nm with poly dispersity index of 0.063 (Figure 7a).

The zeta potential is used to depict the surface charge and stability of synthesized silver nanoparticles using *Pterocarpus indicus*. For leaves-AgNPs zeta potential measured was found to be −13.1 mV with peak area of 100% intensity (Figure 6b). The biosynthesized stem bark-AgNPs had a negative charge with a zeta potential value −16.3 mV (Figure 7b). This zeta potential value falls within the range of −20 to −30 mV is considered as moderately stable, which clearly indicated that the synthesized S-AgNPs was moderately stable in nature (Tables 5 and 6). From the average particle size and PDI value it is found that produced nanoparticles are monodispersed in nature.

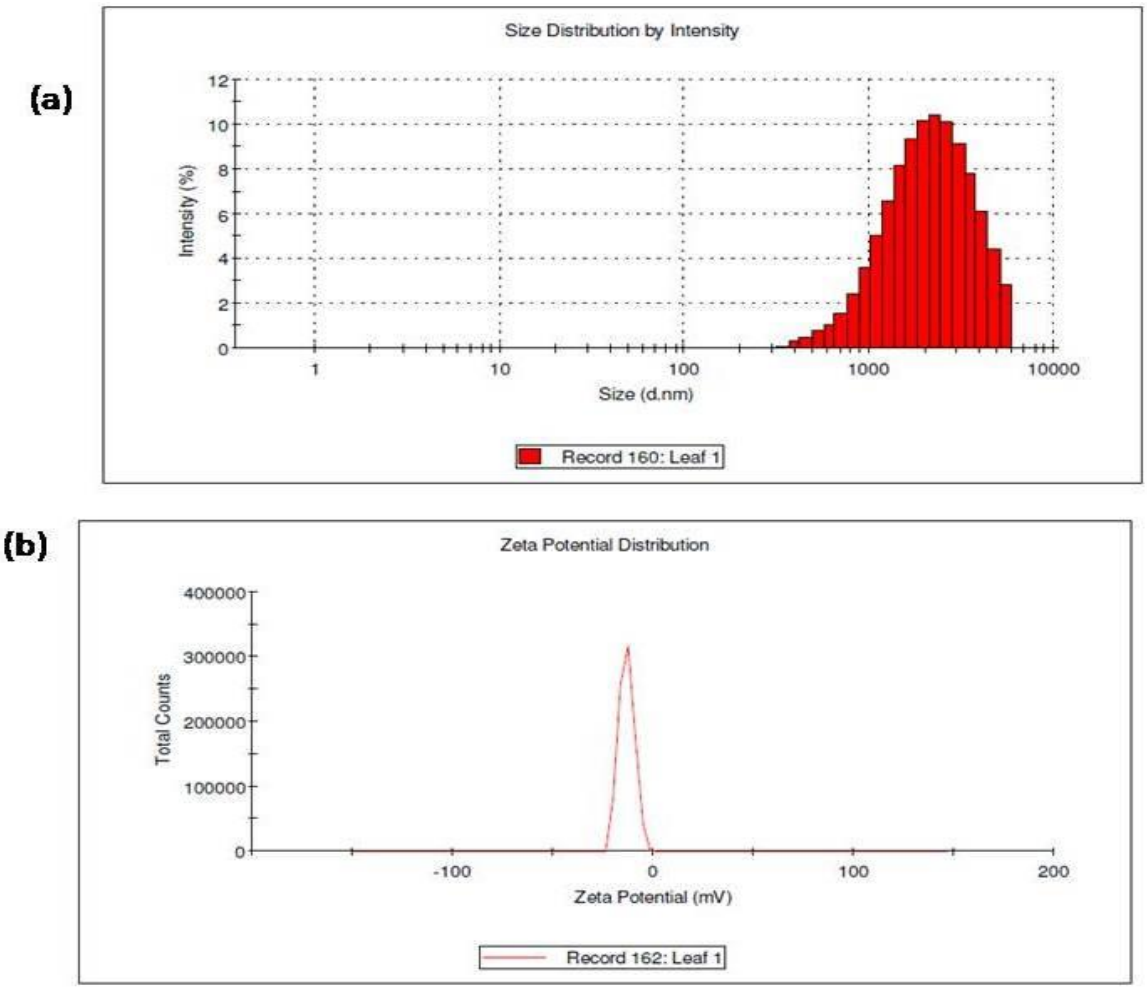



**FIGURE 6(a): Particle Size Distribution and 7(a) Zeta Potential Measurement of the Biosynthesized AgNPs using *Pterocarpus indicus* Leaves Extract**

**TABLE 5: ZETA Potential Analysis of AgNPs using Leaves of *Pterocarpus indicus***

| | | | Size (d. n) | %Intensity | Standard deviation |
|---|---|---|---|---|---|
| Z-Average (d. nm) | 1842 | Peak1 | 2391 | 100.0 | 1201 |
| PdI | 0.247 | Peak2 | 0.000 | 0.0 | 0.000 |
| Intercept | 0.694 | Peak3 | 0.000 | 0.0 | 0.000 |

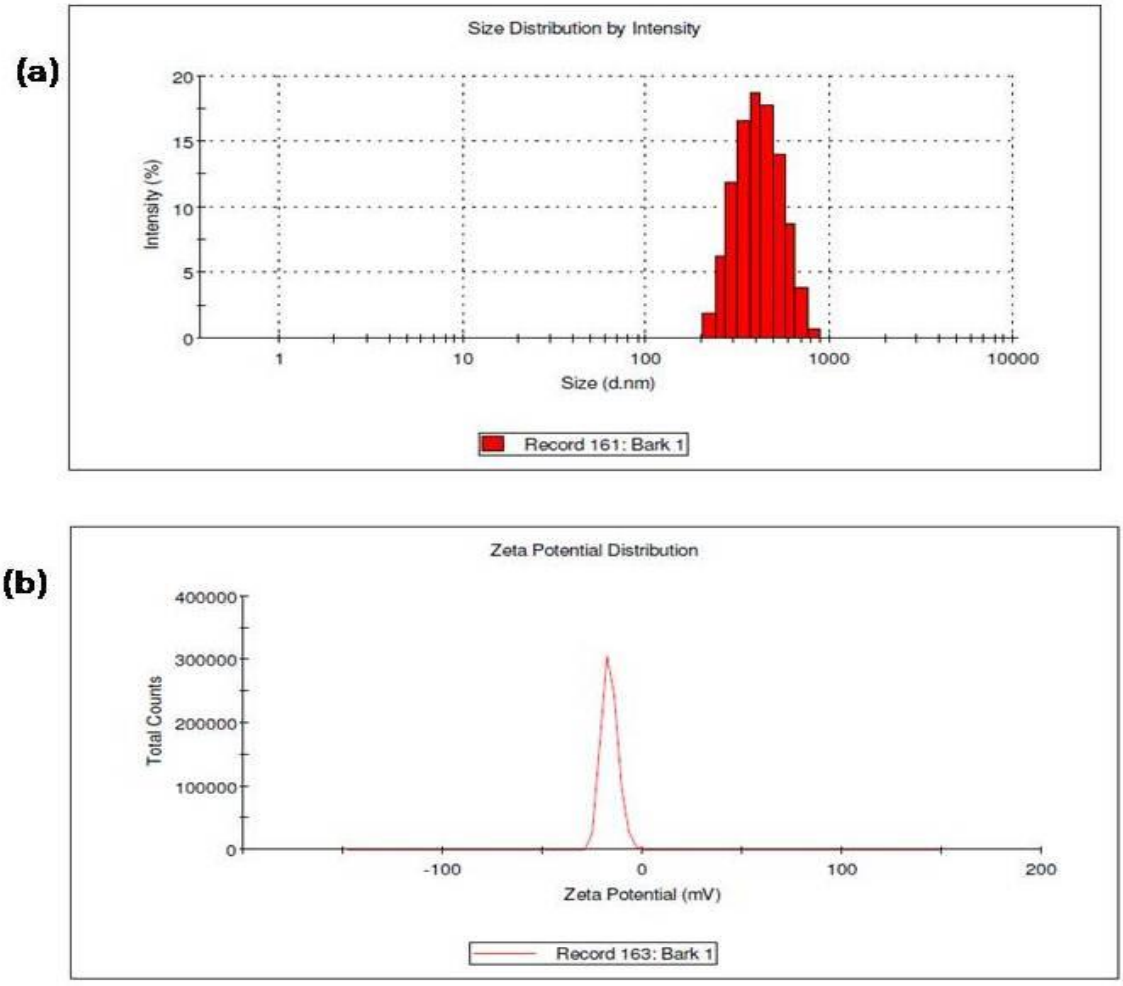



**FIGURE 6(b): Particle Size Distribution and 7(b) Zeta Potential Measurement of the Biosynthesized AgNPs using *Pterocarpus indicus* Stem Bark Extract**

**TABLE 6: Zeta Potential Analysis of AgNPs using Stem Barks of *Pterocarpus indicus***

| | | | Size (d. n) | %Intensity | Standard deviation |
|---|---|---|---|---|---|
| Z-Average (d. nm) | 385.9 | Peak1 | 427.1 | 100.0 | 122.6 |
| PdI | 0.063 | Peak2 | 0.000 | 0.0 | 0.000 |
| Intercept | 0.703 | Peak3: | 0.000 | 0.0 | 0.000 |

## 4.4 Antidiabetic Activity of Synthesized Silver Nanoparticles by using Leaves and Stem Bark of *Pterocarpus indicus* and compared with Standard Drug Acarbose

### 4.4.1 Alpha Amylase Inhibition Assay

The synthesized silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* were investigated by alpha amylase inhibitory activities under *in vitro* conditions (chart 1). The synthesized silver nanoparticles of the various concentrations (20-100 µg/ml) exhibited potent alpha amylase inhibitory activity in a dose dependent manner. The silver nanoparticles using *Pterocarpus indicus* extracts showed inhibitory activity from 56.95% to 67.75% for leaves and for the stem 65.95% to 80.36% at a concentration 100 µg/ml (Table 7). Acarbose is a standard drug and showed the inhibitory activity from 70.27% to 82.88% at a concentration 100 µg/ml has been depicted.

**TABLE 7: *In vitro* Antidiabetic Activity of Silver Nanoparticles Synthesized using *Pterocarpus indicus* Extracts by Alpha Amylase Method and Comparison with Standard Drug Acarbose**

| S. No | Concentration | Alpha amylase (%) | | |
|---|---|---|---|---|
| | | Leaves-AgNPs | Stem Bark-AgNPs | Acarbose |
| 1 | 20 (µg/ml) | 56.04±2.88 | 65.95±4.04 | 70.27±2.78 |
| 2 | 40 (µg/ml) | 59.64±2.28 | 67.75±1.82 | 74.77±3.14 |
| 3 | 60 (µg/ml) | 60.54±2.61 | 73.16±2.49 | 80.18±2.15 |
| 4 | 80 (µg/ml) | 65.95±1.91 | 78.56±3.94 | 81.98±3.26 |
| 5 | 100 (µg/ml) | 67.75±3.56 | 80.36±2.39 | 82.88±3.81 |

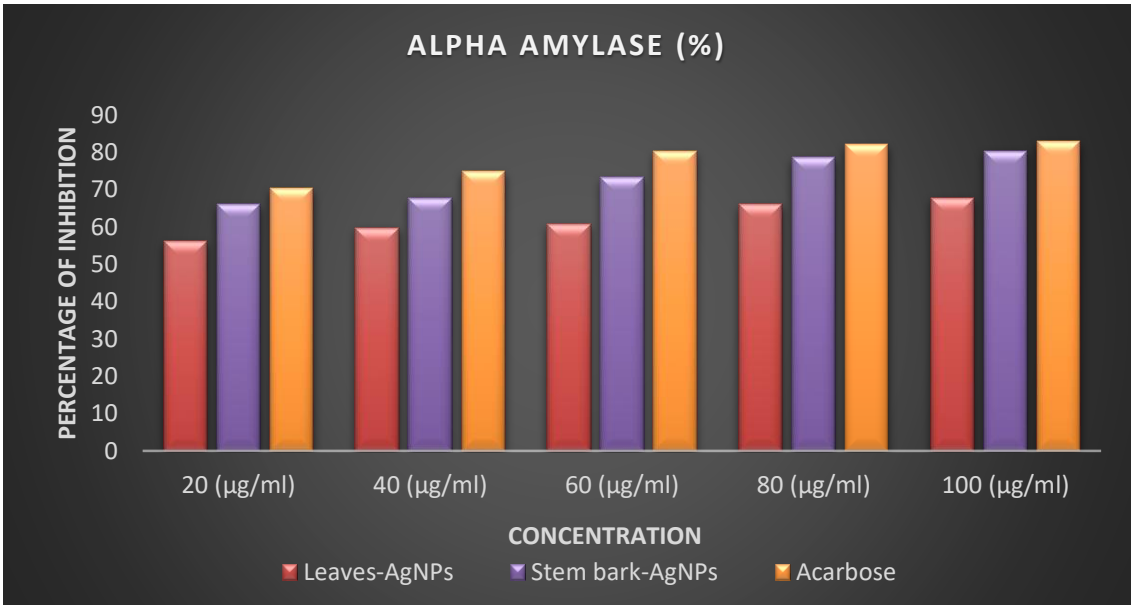



**CHART 1: Alpha Amylase Inhibitory Activity of Acarbose Vs Silver Nanoparticles Synthesized using *Pterocarpus indicus* Extracts**

### 4.4.2 Alpha Glucosidase Inhibitory Assay

The synthesized silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* showed the antidiabetic activity using alpha glucosidase inhibitory activity from 58.74% to 72.85% for leaves and from 48.83% to 64.64% for stem bark at a concentration 100 µg/ml (Table 8). Acarbose is a standard drug at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 70.270% to 82.882%at the same

concentrations 100 µg/ml (chart 2). Thus, the inhibition of the activity of alpha glucosidase by synthesized silver nanoparticles would delay the degradation of carbohydrate, which in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation.

**TABLE 8: *In vitro* Antidiabetic Activity of the Synthesized Silver Nanoparticles by Alpha Glucosidase Method and Comparison with Standard Drug Acarbose**

| S. No | Concentration | Alpha Glucosidase (%) | | |
|---|---|---|---|---|
| | | Leaves-AgNPs | Stem bark-AgNPs | Acarbose |
| 1 | 20 (µg/ml) | 48.83±1.34 | 58.74±2.50 | 70.27±2.78 |
| 2 | 40 (µg/ml) | 50.63±2.66 | 62.35±2.40 | 74.77±3.00 |
| 3 | 60 (µg/ml) | 55.14±2.12 | 64.15±1.35 | 80.18±4.37 |
| 4 | 80 (µg/ml) | 60.74±2.96 | 68.95±2.93 | 81.98±4.80 |
| 5 | 100 (µg/ml) | 64.64±3.88 | 72.85±2.40 | 82.88±3.93 |

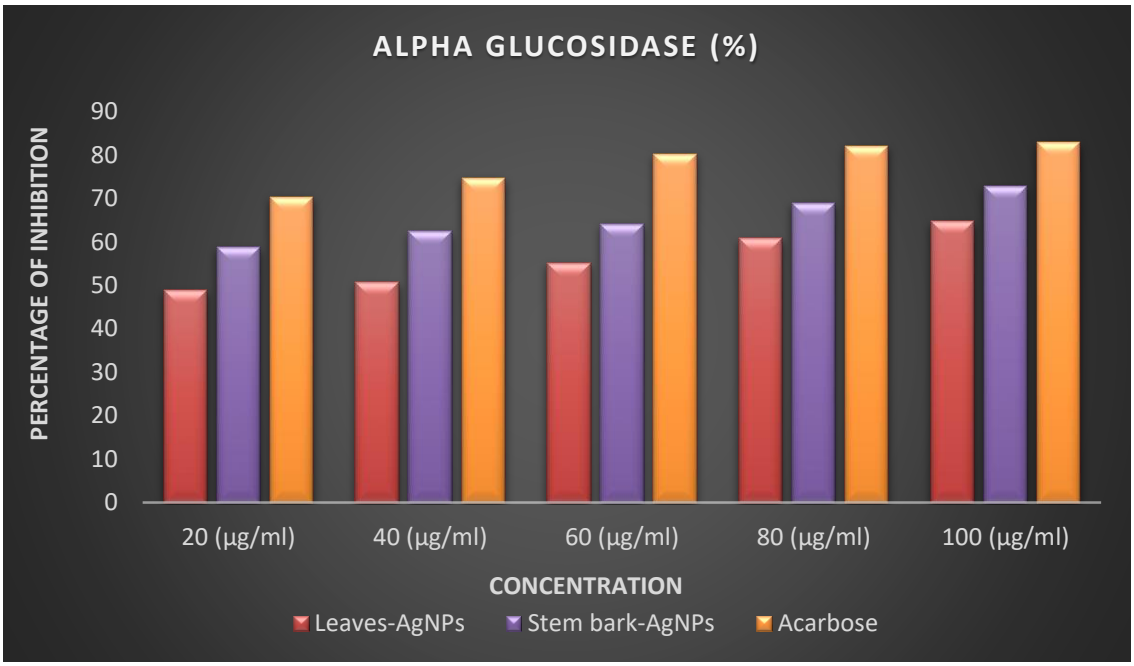



**CHART 2: Alpha Glucosidase Inhibitory Activity of Acarbose Vs Silver Nanoparticles Synthesized using *Pterocarpus indicus***

## 4.5 Cytotoxicity Activity of Synthesized Silver Nanoparticles using Leaves and Stem Bark of *Pterocarpus indicus* on Breast Cancer Cell Line MCF-7

The *in vitro* cytotoxicity study of the biosynthesized silver nanoparticles using leaves and barks of *Pterocarpus indicus* separately were investigated by an MTT assay against MCF-7 breast cancer cell line with different concentration 10-500 µg/ml to determine the IC50 (50% growth inhibition) was summarized in (Table 9). The inhibitory concentration (IC50) value of biosynthesized silver nanoparticles using leaves is 54.60 µg/ml and for the stem barks-AgNPs is 126.4 µg/ml respectively. The table's shows that the stem barks- AgNPs was found more cytotoxic and also showed maximal antiproliferative effect as compared to the leaves-AgNPs on MCF-7 breast cancer cell lines.

The morphological changes of the breast cancer cell lines with various concentrations of leaves-AgNPs and stem bark-AgNPs of *Pterocarpus indicus* at incubation for 24 hours as compared with the untreated cells. After the incubation period, in the treated cancer cells the morphology of the synthesized silver nanoparticles using leaves and stem barks extracts significantly changed and apparent less uniform with the loss of membrane integrity at lower concentrations. Whereas at higher concentrations 400 µg/ml, the synthesized silver nanoparticles treated cells showed significant changes such as karyopyknosis, loss of intact membrane, cell detachment from the plate and changes of morphological features as compared to the control cells without any additive. The leaves-AgNPs and stem bark-AgNPs treated cells most identifiable morphological features of apoptosis were observed by inverted light microscopy. However untreated cells appeared normal and were confluent and for treated cells appeared cells undergoing apoptosis, detaching from the plate, cell shrinkage, condensation, loss of contact with neighbouring cells, and aggregation of the nuclear chromatin.

**TABLE 9: Optical Density and Cell Viability of Synthesized Silver Nanoparticles using Leaves and Stem Bark of *Pterocarpus indicus* on MCF 7 Cell Line by MTT Assay**

| S. No | Tested sample concentration (µg/ml) | Leaves-AgNPs | | | Stem Bark-AgNPs | | |
|---|---|---|---|---|---|---|---|
| | | Absorbance (Optical density) | Cell Viability (%) | ($IC_{50}$) | Absorbance (Optical density) | Cell Viability (%) | ($IC_{50}$) |
| 1. | Control | 0.464 | 100 | **54.60 (µg/ml)** | 0.422 | 100 | **126.4 (µg/ml)** |
| 2. | 10 µg/ml | 0.456 | 98.2 | | 0.348 | 82.61 | |
| 3. | 20 µg/ml | 0.451 | 97.19 | | 0.329 | 78.03 | |
| 4. | 40 µg/ml | 0.434 | 95.04 | | 0.320 | 75.98 | |
| 5. | 60 µg/ml | 0.407 | 92.24 | | 0.302 | 71.72 | |
| 6. | 80 µg/ml | 0.369 | 79.59 | | 0.253 | 59.94 | |
| 7. | 100 µg/ml | 0.318 | 68.6 | | 0.235 | 55.76 | |
| 8. | 200 µg/ml | 0.295 | 63.57 | | 0.196 | 46.52 | |
| 9. | 300 µg/ml | 0.276 | 59.55 | | 0.165 | 39.09 | |
| 10. | 400 µg/ml | 0.219 | 47.19 | | 0.11 | 26.06 | |
| 11. | 500 µg/ml | 0.136 | 29.37 | | 0.095 | 22.58 | |

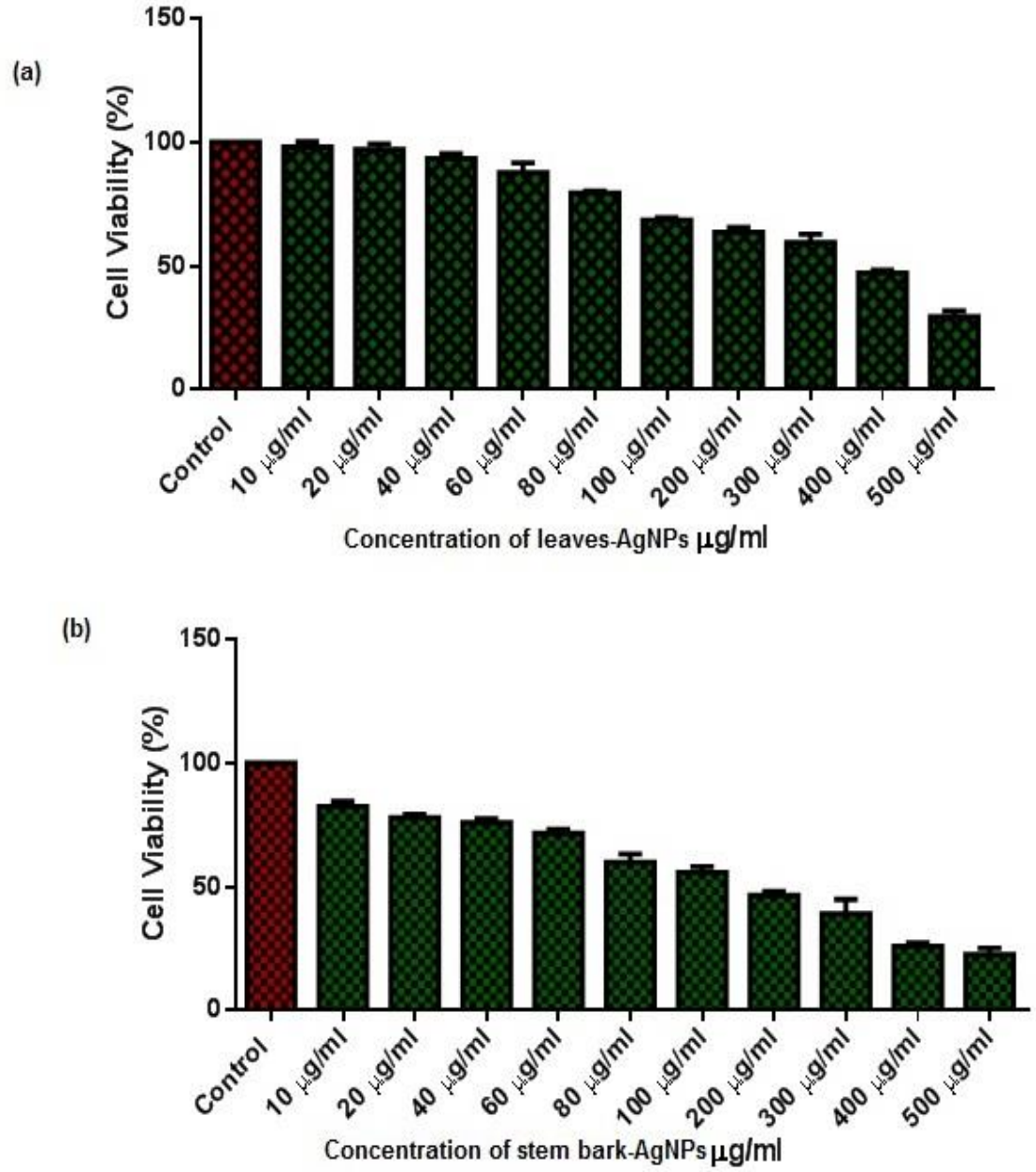



**FIGURE 8: Percentage of Cell Growth Inhibition of Synthesized Silver Nanoparticles using Leaves (a) and Stem Bark (b) of *Pterocarpus indicus* on MCF 7 Cell Line by MTT Assay**

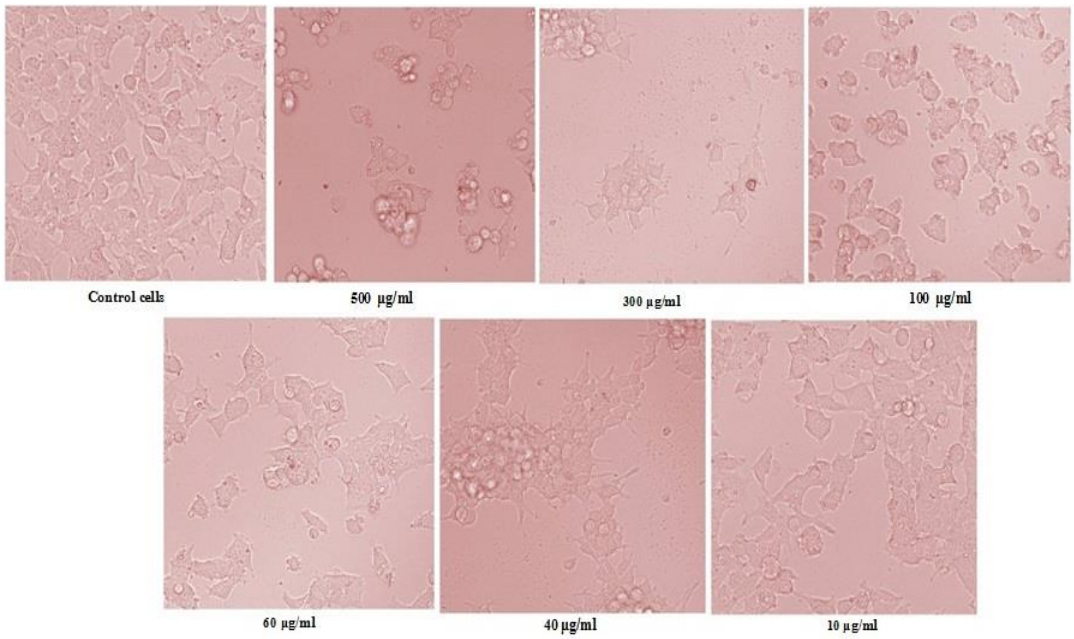



**FIGURE 9: Cytotoxic Effect of Synthesized Silver Nanoparticles using Leaves of *Pterocarpus indicus* on MCF 7 Cell Line by MTT Assay**

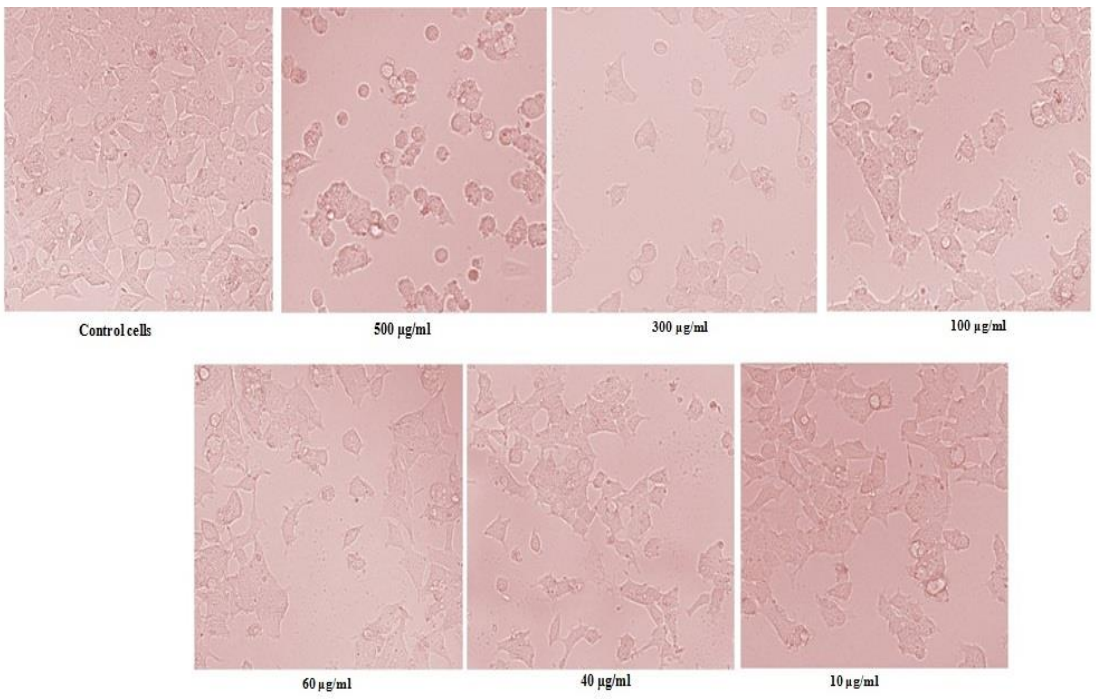



**FIGURE 10: Cytotoxic Effect of Synthesized Silver Nanoparticles using Stem Bark of *Pterocarpus indicus* on MCF 7 Cell Line by MTT Assay**

## 4.6 Antioxidant Activity of Synthesized Silver Nanoparticles using Leaves and Stem Bark of *Pterocarpus indicus*

### 4.6.1 DPPH Assay Method

The result showed that the silver nanoparticles synthesized using a *Pterocarpus indicus* bark and leaf sample of exhibit antioxidant activities at high concentration when compared with standard ascorbic acid (Table 10) and (Chart 3). The synthesized silver nanoparticles showed 70.149% (leaves-AgNPs) and 75.929 (stem bark-AgNPs) activity at concentration 100 µg/ml while ascorbic acid gave 94.69 % at the same concentration.

**TABLE 10: Antioxidant Activity of Silver Nanoparticles Synthesized using *Pterocarpus indicus* by DPPH Activity**

| S. No | Concentration | DPPH Activity% | | |
|---|---|---|---|---|
| | | Leaves-AgNPs | Stem Bark-AgNPs | Ascorbic Acid |
| 1 | 20 (µg/ml) | 55.22±2.29 | 57.76±2.35 | 61.68±2.54 |
| 2 | 40 (µg/ml) | 57.46±2.99 | 62.98±2.80 | 72.85±3.69 |
| 3 | 60 (µg/ml) | 59.70±2.36 | 69.47±2.77 | 79.74±4.82 |
| 4 | 80 (µg/ml) | 64.17±1.62 | 71.46±3.26 | 82.34±2.93 |
| 5 | 100 (µg/ml) | 70.14±2.15 | 75.92±3.98 | 94.69±4.72 |

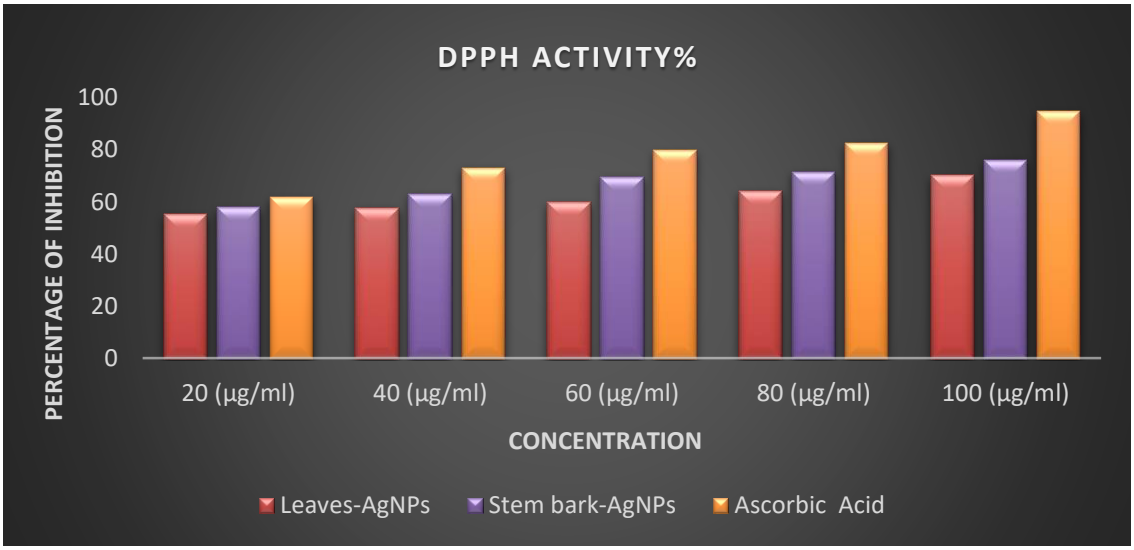



**CHART 3: Antioxidant Activity of Silver Nanoparticles Synthesized using *Pterocarpus indicus* by DPPH Activity**

### 4.6.2 Reducing Power Method

The reducing power of ethanolic extracts of synthesized silver nanoparticles using leaves and bark of *Pterocarpus indicus* was performed and compared with standard ascorbic acid. Ethanolic extract of synthesized stem bark-AgNPs of *Pterocarpus indicus* (78.37%) exhibits good reducing power activity among leaves-AgNPs extracts (79.27%) as we compared to the standard drug ascorbic acid (84.68%) at a concentration 100 µg/ml (Table 11) and (Chart 4).

**TABLE 11: Antioxidant Activity of Stem Barks and Leaves of *Pterocarpus indicus* by Reducing Power Activity**

| S. No | Concentration | Reducing Power Activity % | | |
|---|---|---|---|---|
| | | Leaves-AgNPs | Stem Bark-AgNPs | Ascorbic Acid |
| 1 | 20 (µg/ml) | 52.25±1.12 | 53.15±1.67 | 64.86±2.37 |
| 2 | 40 (µg/ml) | 56.75±1.73 | 61.26±1.83 | 66.66±3.36 |
| 3 | 60 (µg/ml) | 68.46±3.33 | 67.56±2.61 | 70.27±3.36 |
| 4 | 80 (µg/ml) | 73.81±4.35 | 75.67±3.77 | 81.08±3.14 |
| 5 | 100 (µg/ml) | 78.37±4.29 | 79.27±4.29 | 84.68±4.97 |

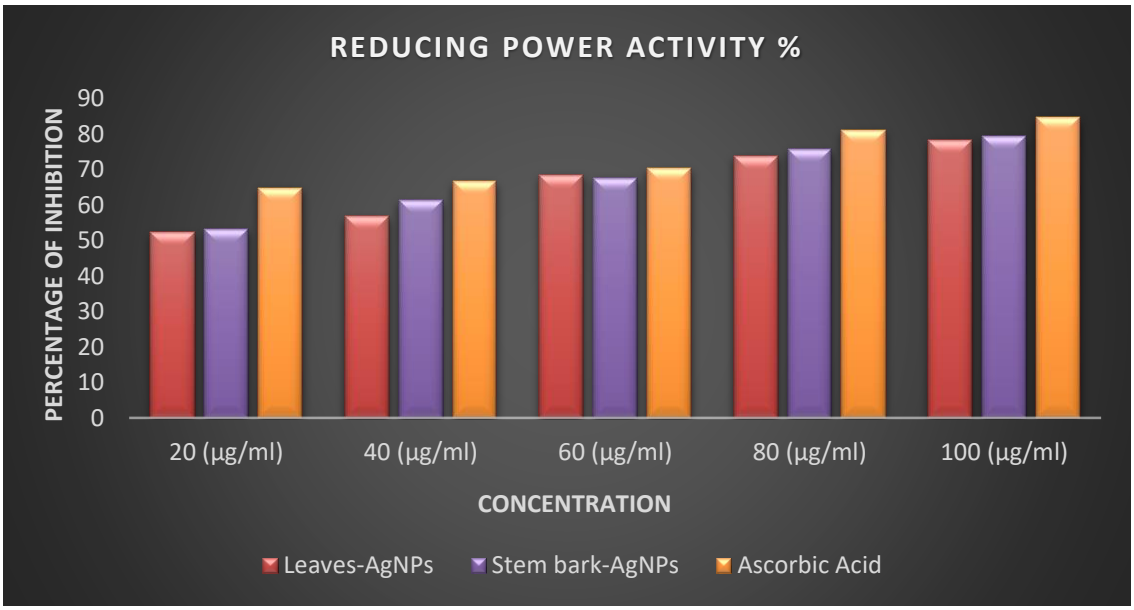



**CHART 4: Antioxidant Activity of Stem Bark and Leaves of *Pterocarpus indicus* by Reducing Power Activity**

## 4.7 Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds from the Leaves and Stem Bark of *Pterocarpus indicus*

GCMS is an important tool for the identification of bioactive compounds present in the leaves and bark of *Pterocarpus indicus* and also used to analysis their retention time, molecular formulae, molecular weight and structure of the active constituents present in the extracts (Table 12 and 13). So, in the present study, 10 bioactive compounds were identified from the ethanolic extracts of leaves (Figure 11) and 20 bio active compounds from the stem barks of *Pterocarpus indicus* (Figure 12).

**TABLE 12: Bioactive Compounds detected in Ethanolic Leaves Extract of *Pterocarpus indicus* by GCMS Analysis**

| S. No | R. Time | Area% | Height% | Compound Name | Molecular Formula | Molecular Weight |
|---|---|---|---|---|---|---|
| 1 | 14.031 | 3.31 | 8.16 | Docosane | $C_{22}H_{46}$ | 310 |
| 2 | 15.609 | 48.92 | 33.61 | Phthalic acid, di-(1-hexen-5-yl) ester | $C_{20}H_{26}O_4$ | 330 |
| 3 | 15.842 | 5.36 | 13.35 | Propanoyl chloride | $C_3H_5ClO$ | 92 |
| 4 | 15.967 | 9.47 | 8.19 | Methane, Thiobis- | $C_2H_6S$ | 62 |
| 5 | 16.092 | 7.72 | 7.63 | 1,3-dioxolane,2-(phenylmethyl)- | $C_{10}H_{12}O_2$ | 164 |
| 6 | 16.283 | 3.45 | 2.51 | n- Allyloxymethyl acrylamide | $C_7H_{11}NO_2$ | 141 |
| 7 | 21.084 | 3.59 | 7.53 | Silane,[1-(5-hexenyl)-2methylenecyclopropy | $C_{13}H_{24}S_i$ | 208 |
| 8 | 21.236 | 11.29 | 10.13 | 14-Heptadecenal | $C_{17}H_{32}O$ | 252 |
| 9 | 23.518 | 3.44 | 3.28 | 4,6-Decadiyn-3-ol,3-isopropyl-9-(methoxyeth | $C_{17}H_{28}O_4$ | 296 |
| 10 | 25.058 | 3.44 | 5.62 | Silicate anion tetramer | $C_{24}H_{72}O_{12}S_{i12}$ | 888 |
| | | 100.00 | 100.00 | | | |

**TABLE 13: Bioactive Compounds Identified in Ethanolic Bark Extract of *Pterocarpus indicus* by GC-MS Analysis**

| S. No | R. Time | Area % | Height% | Compound Name | Molecular Formula | Molecular Weight |
|---|---|---|---|---|---|---|
| 1 | 5.293 | 5.55 | 3.77 | 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl | $C_8H_{14}O$ | 126 |
| 2 | 5.447 | 1.83 | 1.85 | 1,2,3-Propanetriol, diacetate (CAS) Diacetin | $C_7H_{12}O_5$ | 176 |
| 3 | 7.851 | 23.55 | 14.02 | Cytidine (CAS) Cyd | $C_9H_{13}N_3O_5$ | 243 |
| 4 | 8.689 | 0.87 | 1.51 | Zingiberene | $C_{15}H_{24}$ | 204 |
| 5 | 9.332 | 0.69 | 1.02 | Hexadecanoic acid (CAS) Palmitic acid | $C_{16}H_{32}O_2$ | 256 |
| 6 | 9.944 | 0.85 | 1.25 | Benzoldicarbonsaeure | $C_{20}H_{26}O_4$ | 330 |
| 7 | 11.708 | 1.27 | 1.87 | Dodecanoic acid (CAS) Lauric acid | $C_{12}H_{24}O_2$ | 200 |
| 8 | 12.600 | 1.51 | 2.20 | Cyclopentane, heneicosyl- (CAS) Heneicosane | $C_{26}H_{52}$ | 364 |
| 9 | 13.076 | 1.48 | 2.02 | 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | $C_9H_{18}O$ | 142 |
| 10 | 13.708 | 1.21 | 0.51 | 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | $C_{11}H_{17}NO_4$ | 227 |
| 11 | 13.894 | 12.50 | 14.92 | Nonadecanoic Acid | $C_{19}H_{38}O_2$ | 298 |
| 12 | 14.100 | 2.28 | 0.94 | Phthalic acid, butyl ester, ester with butyl glyc | $C_{18}H_{24}O_6$ | 336 |
| 13 | 14.760 | 1.49 | 2.18 | Bi-1,3,5-cycloheptatrien-1-yl (CAS) | $C_{14}H_{12}$ | 180 |
| 14 | 14.996 | 21.33 | 31.11 | 5,8,11,14-Icosatetraynoic Acid | $C_{20}H_{24}O_2$ | 296 |
| 15 | 15.508 | 1.82 | 2.44 | dl-Citronellol | $C_{10}H_{20}O$ | 156 |
| 16 | 15.732 | 3.22 | 3.32 | 1, E-11, Z-13-Octadecatriene | $C_{18}H_{32}$ | 248 |
| 17 | 15.816 | 7.04 | 6.65 | 9,12,15-Octadecatrienoic acid, methyl ester | $C_{19}H_{32}O_2$ | 292 |
| 18 | 15.979 | 2.51 | 2.55 | 9-Octadecenoic acid (Z)- (CAS) Oleic acid | $C_{18}H_{34}O_2$ | 282 |
| 19 | 19.328 | 2.91 | 2.05 | Trans-2-Phenyl-1,3-Dioxolane-4-M | $C_{28}H_{40}O_4$ | 440 |
| 20 | 22.781 | 6.11 | 3.79 | 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | $C_{24}H_{38}O_4$ | 390 |
| | | 100.00 | 100.00 | | | |

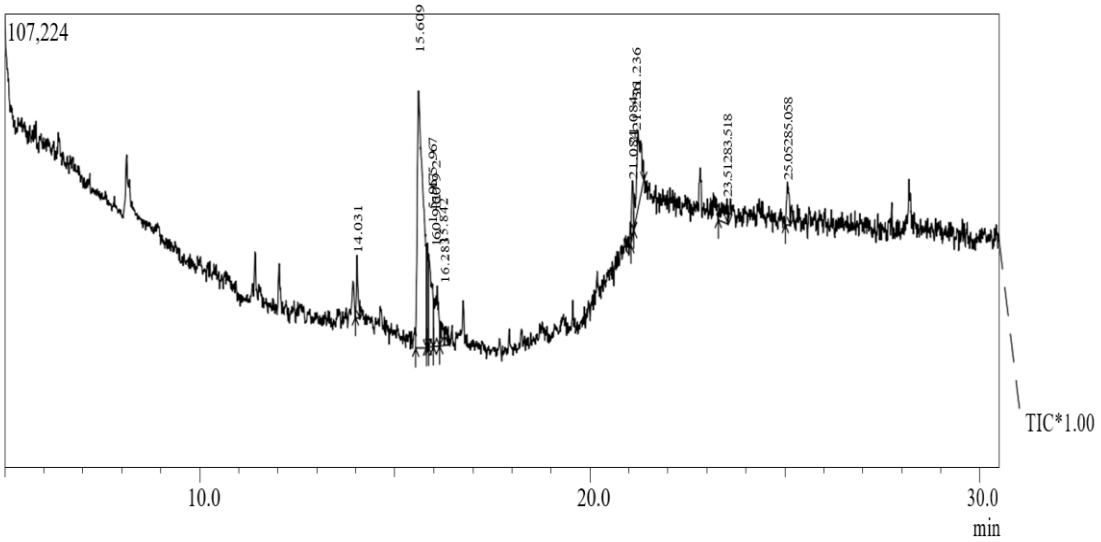



**FIGURE 11: GC-MS Chromatogram of the Ethanolic Extract of *Pterocarpus indicus* Leaves**

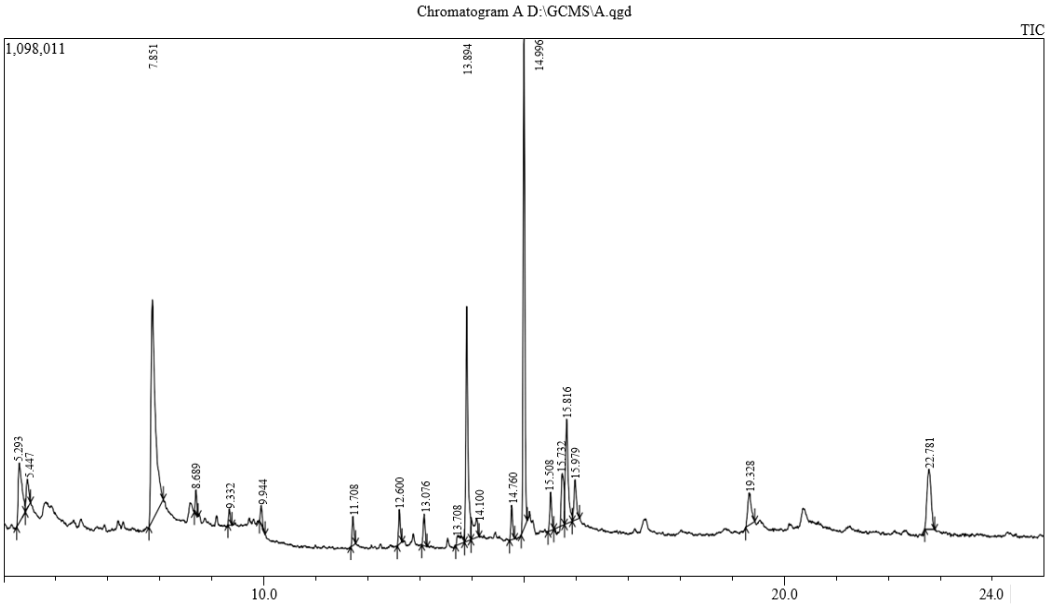



**FIGURE 12: GC-MS Chromatogram of the Ethanolic Extract of *Pterocarpus indicus* Bark**

## 4.8 *In-silico* Study of Phyto Compounds Identified from the Leaves and Barks of *Pterocarpus indicus* for Alpha amylase and Alpha glucosidase Inhibitory Activity

### 4.8.1 Molecular Dockings Analysis for Alpha Amylase

Docking studies by Maestro (Schrodinger) showed that the compounds cytidine (CAS) cyd had the highest docking score (-7.998) followed by 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon (-7.222), benzoldicar bonsaeure (-6.093), 1,2-benzenedicarboxylic acid, bis (2-ethylhexy) (-5.689) against alpha amylase enzymes acarbose which is -14.339 KJ/MOL which may be a potent anti-diabetic compounds because the high docking score, the compound cytidine (CAS) cyd will be potent antidiabetic drugs against alpha amylase activity (Table 14). Acarbose docking with alpha amylase indicted that the model structure of ligand-protein interaction between acarbose and alpha amylase had formed interaction of ligand molecules (Figure 13) showed for the compound's Phthalic acid, butyl ester, ester with butyl glyc (GLN63. TYR 62, TRP 59, TRP 58, ALA 198, ASP 197, ARG 195, GLU 233, ILE 235, ASP 300, LEU 165, VAL 163, LEU 162), Cytidine (CAS) Cyd (TRP 58, TRP 59, GLU 60, TYR 62, GLN 63, ARG 195, ASP 300, HIS 299, HIP 305), Bi-1,3,5-cycloheptatrien-1-yl (GLN 63, TYR 62, TRP 59, TRP 58, ASP 300, HIS 299, ARG 195, ASP 197, ALA 198, Phthalic acid, di-(1-hexen-5-yl) ester (HIP 305, GLY 305, TRP 58, TRP 59, TYR 62, GLN 63, ALA 198, ASP 197, ARG 195, GLU 233), 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon (HIP 305,GLY 306, GLU 233, ASP 300, HIS 299, TYR 62, GLN 63, TRP 59, TRP 58, LEU 162, VAL 163, LEU 165, ALA 198, ASP 197, ARG 195, Silane, [1-(5-hexenyl)-2methylenecyclopropy (TRY 62, HIE 101, TRP 59, TRP 58, ALA 198, ASP 197, ARG 195, ASP 300, HIS 299, ILE 235, GLU 233, GLY 306, HIP 305, LEU 162, VAL 163, LEU 165), Zingiberene (HIP 305, TRP 58, TRP 59, TYR 62, GLN 63, ASP 300, HIS 299, LEU 165, VAL 163, LEU 162. GLU 233), Cyclopentane, heneicosyl- (CAS) Heneicosane (ALA 107, ALA 108, GLY 104, HIE 101, ARG 195, ASP 197, ALA 198,

LYS 200, HIS 201, TYR 151, GLN 63, TYR 62, TRP 59, GLU 233, ILE 235), 1,3-dioxolane, 2-(phenylmethyl)- (GLN 63, TYR 62, ASP 300, TRP 59, TRP 58, VAL 163, LEU 165, HIE 101), Benzoldicarbonsaeure (GLY 306, HIP 305, TRP 58, TRP 59, TYR 62, GLN 63, LEU 165, VAL 163, LEU 162), dl-Citronellol (ARG 195, ASP 197, ALA 198, LEU 162, VAL 163, LEU 165, GLN 63, TYR 62, HIE 101, TRP 59, TRP 58, ASP 300, HIS 299), 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol (ARG 195, ASP 197, ALA 198, HIS 299, ASP 300, TRP 58, TRP 59, TYR 62, GLN 63), 5,8,11,14-Icosatetraynoic Acid (LEU 165, GLY 164, VAL 163, LEU 162, GLN 63, TYR 62, TRP 59, TRP 58, ASP 300, HIS 299, VAL 98, HIE 101, GLY 104, SER 105, GLY 106, ALA 107, VAL 51), Nonadecnoic Acid (HIS 201, LYS 200, ALA 198, ASP 197, ARG 195, GLN 63, TYR 62, TRP 59, TRP 58, , TYR 151, LEU 162, VAL 163, LEU 165), 1, E-11, Z-13-Octadecatriene (TRP 58, TRP 59, TYR 62, GLN 63, ARG 195, ASP 197, ALA 198, LYS 200, HIS 201, LEU 165, VAL 163, LEU 162), Docosane (GLN 63, TYR 62, TRP 59, TRP 58, GLY 306, GLU 233, VAL 234, ILE 235, HIS 201, LYS 200, SER 199, ALA 198, ASP 197, ARG 195, ALA 107, GLY 104, HIE 101), Hexadecanoic acid (CAS) Palmitic acid (TRP 58, TRP 59, TYR 62, GLN 63, LEU 165, VAL 163, LEU 162, HIP 305, GLY 306, ASP 300,ASP 356,TRP 357), 9-Octadecenoic acid (Z)- (CAS) Oleic acid (TRP 59, GLU 60, TYR 62,GLN 63, ALA 198, ASP 197, ARG 195, GLU 233, ILE 235, HIP 305, GLY 306, LEU 162, VAL 163, LEU 165), 4,6-Decadiyn-3-ol, 3-isopropyl-9-(methoxyeth (GLN 63, TYR 62, TRP 59, TRP 58, LEU 165, VAL 163, LEU 162, HIS 299, ASP 300, ARG 195, ASP 197, ALA 198, HIS 201, GLY 104, ALA 107), 14-Heptadecenal (GLU 233, VAL 234, ILE 235, HIS 201, LYS 200, ALA 198, ASP 197, ARG 195, ASP 300, HIS 299, HIP 305, GLY 306, TYR 151, HIE 101), 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl (TRP 58, TRP 59, TYR 62,GLN 63, LEU 165, VAL 163,LEU 163, LEU 162, HIE 101, ALA 198, HIP 305), 1,2,3-Propanetriol, diacetate (CAS) Diacetin (GLN 63, TYR 62, GLU 60, TRP 59, TRP 58, ASP 300, LEU 165, VAL 163,LEU 162), Propanoyl chloride (ASP 300, HIS 299, TRP 58, ARG 195, ASP 197,

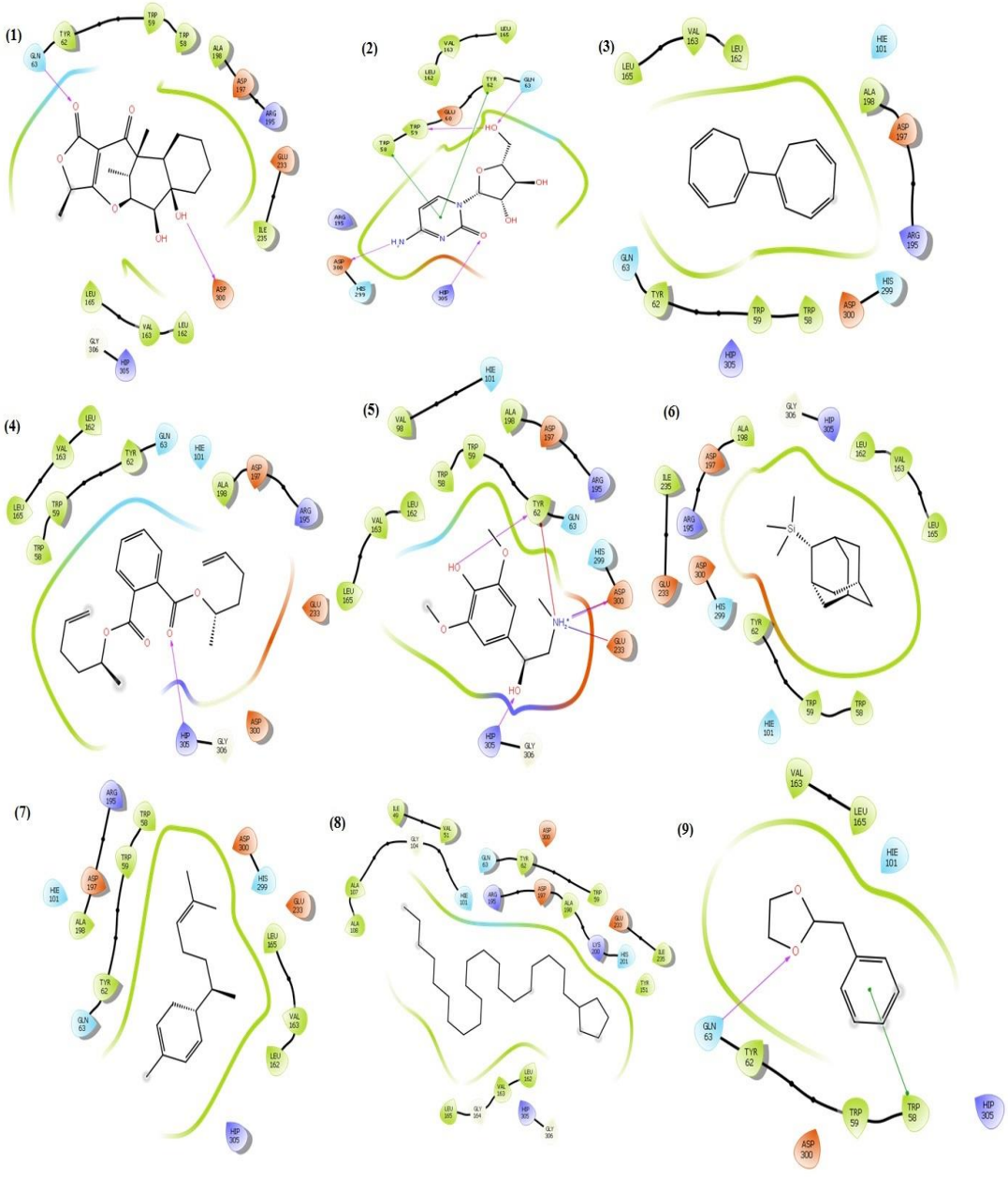
ALA 198, LYS 200, HIS 201, GLU 233, ILE 235, LEU 237, GLU 240, GLY 306, ALA 307, LEU 165, VAL 163, LEU 162), Methane, Thiobis- (GLU 272, LYS 261, ASN 279, TYR 276, TRP 284, GLY 283), n- Allyloxymethyl acrylamide (GLN 63, TYR 62, TRP 59, LEU 162, VAL 163, LEU 165, ARG 195, ASP 197, ALA 198), Dodecanoic acid (CAS) Lauric acid (LEU 162, VAL 163, GLY 164, LEU 165, HIP 305, GLY 306, GLN 63, TYR 62, TRP 59, TRP 58), 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy (TRP 58, TRP 59, TYR 62, GLN 63, ALA 107, GLY 106, SER 105, GLY 104, HIE 101, LEU 162, VAL 163, GLY 164, LEU 165, HIS 299, ARG 195, HIS 201, LYS 200), Trans-2-Phenyl-1,3-Dioxolane-4-M (HIP 305, GLY 306, ALA 307, TRP 59,58, ARG 195, ASP 197, ALA198, LYS 200, HIS 201, HIE 101, HIS 299, ASP 300), 9,12,15-Octadecatrienoic acid, methyl ester (TRP 58, ARG 195, ASP 197, ALA 198, LYS 200, HIS 201, GLU 233, ILE 235, LEU 237, LEU 165, VAL 163, LEU 162, GLY 306, ALA 307). Acarbose TRP 58, TRP 59, GLU 60, TYR 62, GLN 63, ARG 195, ASP 197, ALA 198, LYS 200, HIS 201, GLU 240, LEU 165, VAL 163, LEU 162, VAL 98, ASN 100, HIE 101).

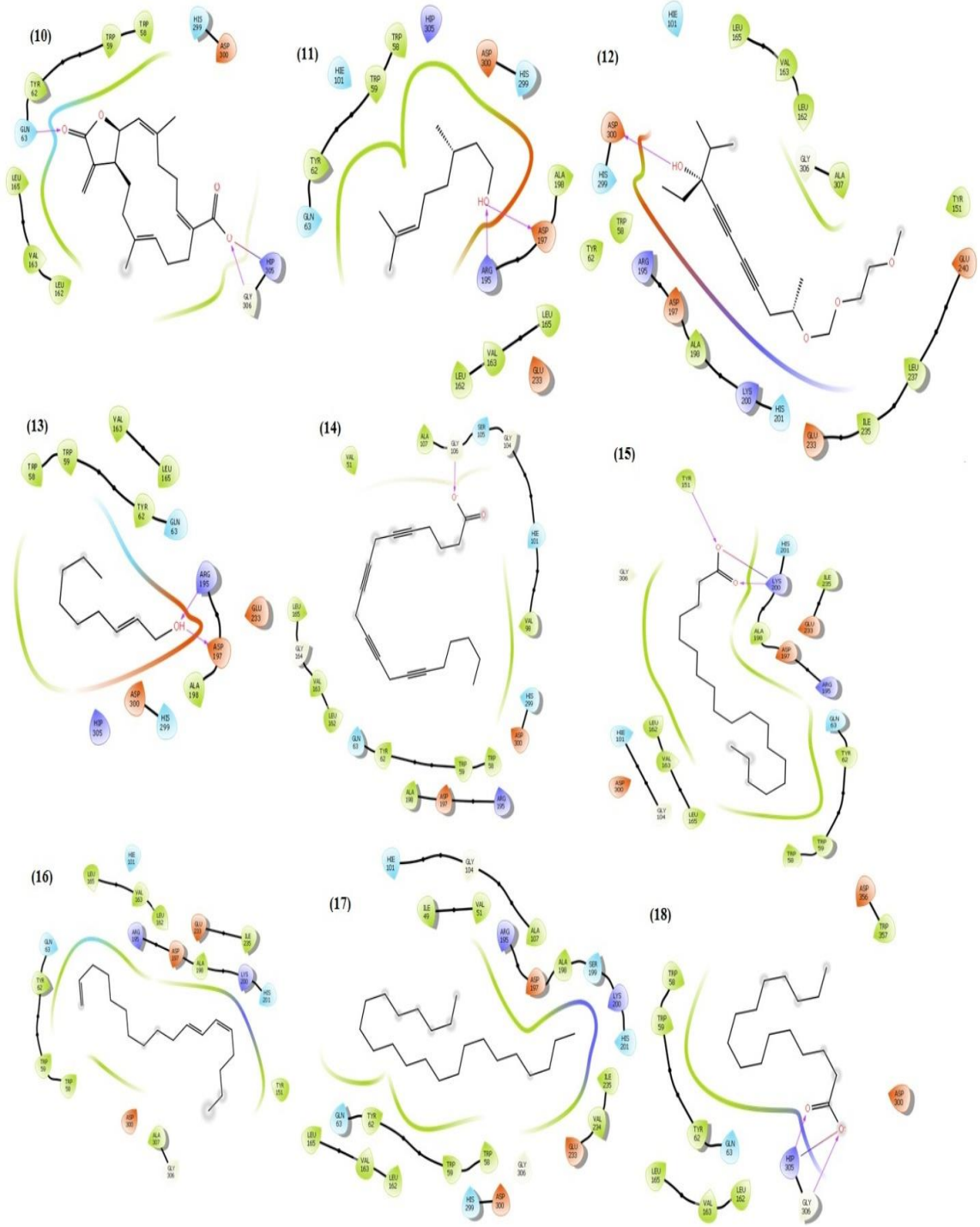
**TABLE 14: Docking Results of Bioactive Compounds from Leaves and Barks of *Pterocarpus indicus* in Alpha Amylase Enzymes (PDB: 1PP1)**

| SI. No | CID | Compound name | Glide score | Glide Evdw | Glide Ecoul | Glide Energy | Glide einternal | Glide emodel |
|---|---|---|---|---|---|---|---|---|
| 1. | 41774 | Acarbose | -14.339 | -41.796 | -39.267 | -81.063 | 11.13 | -103.252 |
| 2. | 6175 | Cytidine (CAS) Cyd | -7.998 | -20.438 | -13.725 | -34.163 | 8.691 | -41.965 |
| 3. | 31513 | 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | -7.222 | -20.406 | -14.787 | -35.193 | 2.728 | -43.382 |
| 4. | 3074876 | Phthalic acid, butyl ester, ester with butyl glyc | -6.638 | -32.986 | -5.848 | -38.833 | 0.634 | -48.555 |
| 5. | 5358705 | Benzoldicarbonsaeure | -6.093 | -19.4 | -7.152 | -26.552 | 1.748 | -35.744 |
| 6. | 5387599 | Trans-2-Phenyl-1,3-Dioxolane-4-M | -5.827 | -39.347 | -4.878 | -44.225 | 0 | -63.06 |
| 7. | 7057919 | 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | -5.689 | -38.081 | -2.555 | -40.636 | 3 | -58.916 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8. | 66021 | 1,2,3-Propanetriol, diacetate (CAS) Diacetin | -5.609 | -13.277 | -13.867 | -27.144 | 7.343 | -31.025 |
| 9. | 59029 | Phthalic acid, di-(1-hexen-5-yl) ester | -4.76 | -35.911 | -4.589 | -40.5 | 1.685 | -56.915 |
| 10. | 5362893 | 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl | -4.582 | -13.97 | -3.042 | -17.012 | 2.919 | -17.823 |
| 11. | 7562 | 1,3-dioxolane, 2-(phenylmethyl)- | -4.332 | -18.126 | -1.842 | -19.968 | 3.424 | -24.074 |
| 12. | 563003 | Bi-1,3,5-cycloheptatrien-1-yl (CAS) | -4.168 | -25.94 | -1.158 | -27.098 | 0.318 | -35.05 |
| 13. | 23171 | Cyclopentane, heneicosyl- (CAS) Heneicosane | -3.83 | -35.56 | -0.219 | -35.778 | 3.892 | -44.235 |
| 14. | 92776 | Zingiberene | -3.719 | -22.695 | 0.143 | -22.553 | 0.663 | -28.546 |
| 15. | 547946 | 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth | -3.586 | -26.004 | -8.84 | -34.844 | 5.796 | -43.589 |
| 16. | 62324 | Propanoyl chloride | -3.341 | -10.862 | -2.823 | -13.685 | 2.638 | -14.465 |
| 17. | 8842 | dl-Citronellol | -3.303 | -13.783 | -7.658 | -21.441 | 4.345 | -25.184 |
| 18. | 13131343 | Silane, [1-(5-hexenyl)-2methylenecyclopropy | -3.005 | -26.277 | -0.364 | -26.641 | 0.04 | -33.387 |
| 19. | 5364941 | 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | -2.993 | -11.248 | -10.309 | -21.557 | 4.087 | -24.374 |
| 20. | 1780 | 5,8,11,14-Icosatetraynoic Acid | -2.61 | -29.766 | -4.967 | -34.734 | 2.136 | -41.989 |
| 21. | 5367462 | 9,12,15-Octadecatrienoic acid, methyl ester | -2.547 | -30.114 | -3.237 | -33.351 | 4.168 | -43.492 |
| 22. | 445639 | 9-Octadecenoic acid (Z)- (CAS) Oleic acid | -2.419 | -24.847 | -4.867 | -29.715 | 2.736 | -35.014 |
| 23. | 12591 | Nonadecnoic Acid | -2.107 | -25.854 | -3.195 | -29.05 | 2.476 | -31.7 |
| 24. | 985 | Hexadecanoic acid (CAS) Palmitic acid | -1.845 | -19.862 | -6.073 | -25.935 | 2.215 | -28.261 |
| 25. | 12405 | Docosane | -1.529 | -29.249 | 0.112 | -29.137 | 1.38 | -43.124 |
| 26. | 5367667 | 14-Heptadecenal | -1.406 | -30.092 | -4.158 | -34.25 | 13.124 | -37.8 |
| 27. | 1068 | Methane, Thiobis- | -1.341 | -6.013 | -2.32 | -8.332 | 0 | -9.188 |
| 28. | 3893 | Dodecanoic acid (CAS) Lauric acid | -1.244 | -16.082 | -5.862 | -21.945 | 1.731 | -22.295 |
| 29. | 5365585 | 1, E-11, Z-13-Octadecatriene | -0.925 | -25.002 | -0.82 | -25.822 | 3.271 | -30.159 |
| 30. | 19357475 | n- Allyloxymethyl acrylamide | 0.122 | -17.884 | -3.347 | -21.23 | 3.643 | -24.235 |

**Figure 13: Molecular docking analysis of 1)Phthalic acid, butyl ester, ester with butyl glyc 2)Cytidine (CAS) Cyd 3) Bi-1,3,5-cycloheptatrien-1-yl (CAS) 4)Phthalic acid, di-(1-hexen-5-yl) ester 5) 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon 6) Silane, [1-(5-hexenyl)-2methylenecyclopropy 7) Zingiberene 8) Cyclopentane, heneicosyl- (CAS) Heneicosane 9) 1,3-dioxolane, 2-(phenylmethyl)- 10) Benzoldicarbonsaeure 11) dl-Citronellol 12) 9,12,15-Octadecatrienoic acid, methyl ester 13) 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol 14) 5,8,11,14-Icosatetraynoic Acid 15) Nonadecnoic Acid 16) 1, E-11, Z-13-Octadecatriene 17)Docosane 18) Hexadecanoic acid (CAS) Palmitic acid 19) 9-Octadecenoic acid (Z)-(CAS) Oleic acid 20)4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth 21)14-Heptadecenal 22) 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl 23) 1,2,3-Propanetriol, diacetate (CAS) Diacetin 24) Propanoyl chloride 25)Methane, Thiobis-26) n- Allyloxymethyl acrylamide 27) Dodecanoic acid (CAS) Lauric acid 28) 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy 29) Trans-2-Phenyl-1,3-Dioxolane-4-M 30) Acarbose with alpha amylase enzymes (PDB 1PP1) obtained from GLIDE docking.**

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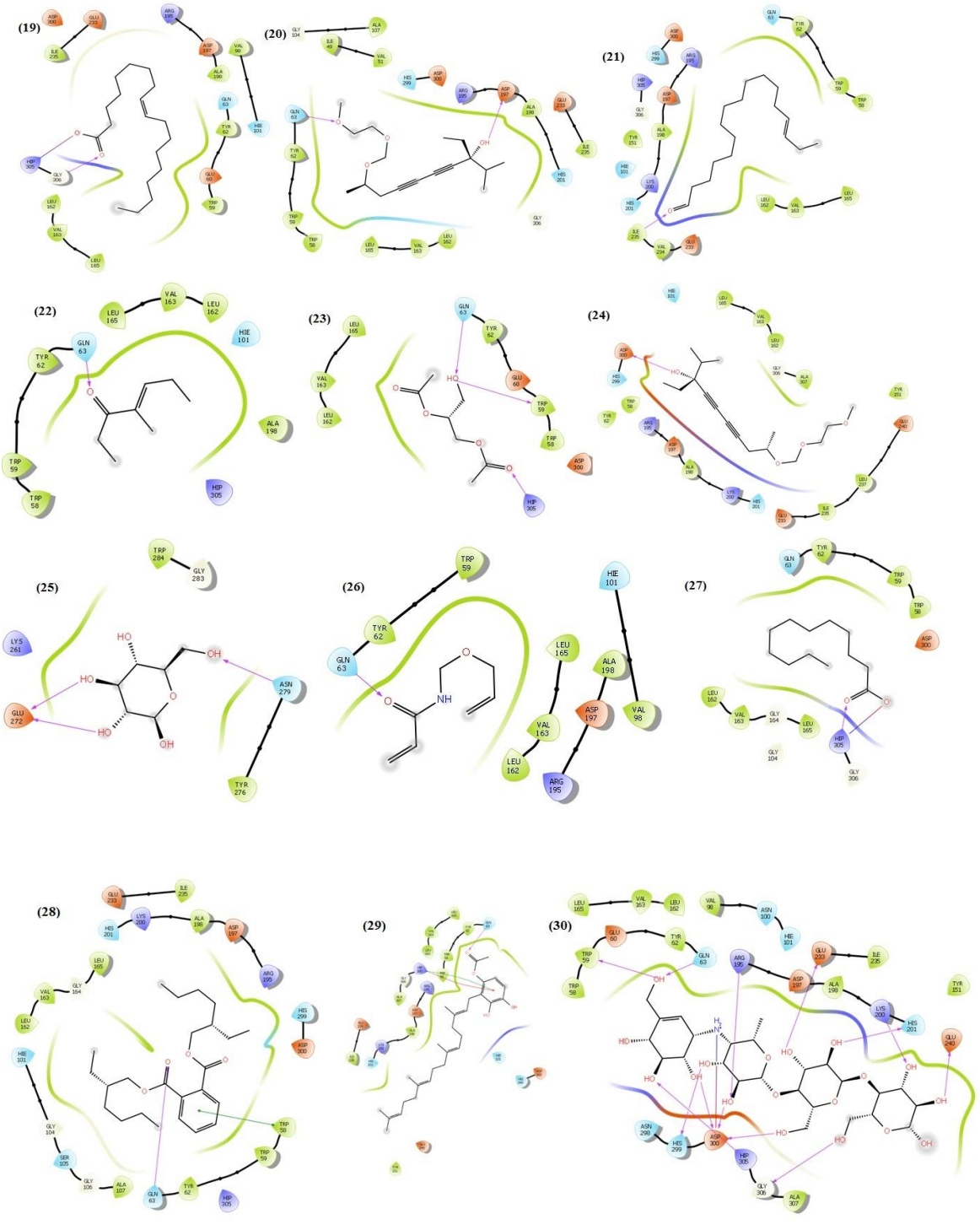
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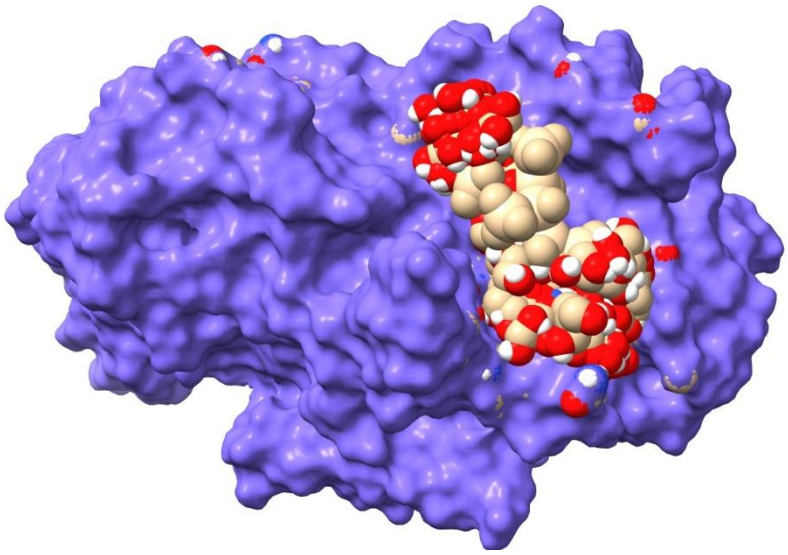


**FIGURE: 14 Compound Cytidine (CAS) Cyd Docked with the Binding site of Alpha Amylase**

### 4.8.2. Molecular Dockings Analysis for Alpha Glucosidase

Docking studies by Maestro (Schrodinger) showed that the compounds 2-(4-hydroxyl-2-butenyl)-2-nitrocyclo heptanon (-7.496) followed by trans-2-phenyl-1,3-dioxolane-4 (-6.472), cytidine (CAS) cyd (-6.233), phthalic acid, butyl ester, ester with butyl glyc (-5.036) against alpha glucosidase enzymes acarbose which is -14.452 KJ/MOL which may be a potent anti-diabetic compounds because the high docking score, the compound 2-(4-hydroxyl-2-butenyl)-2-nitrocyclo heptanon will be potent antidiabetic drugs against alpha glucosidase activity (Table 15). Acarbose docking with alpha glucosidase indicted that the model structure of ligand-protein interaction between acarbose and alpha glucosidase had formed interaction of ligand molecules (Figure 15) showed for the compounds Phthalic acid, butyl ester, ester with butyl glyc (ASP 282, ARG 600, ASP 616, PHE 525, SER 523, TRP 481, ILE 441, LEU 650, PHE 649, LEU 405, ASP 404, TRP 376), Cytidine (CAS) Cyd ( ASP 616, ARG 600, TRP 613, HIS 674, ARG 672, TRP 376, ILE 441, ASP 404, LEU 405, TRP 516, ASP 518, MET 519, SER

523, ASP 282, PHE 525), Bi-1,3,5-cycloheptatrien-1-yl (CAS) (PHE 525, ASP 282, MET 519, ASP 518, TRP 516, ILE 441, ASP 404, LEU 405, TRP 376, TRP 481, ASP 616, TRP 613), Phthalic acid, di-(1-hexen-5-yl) ester (PHE 525, ASN 524, SER 523, TRP 481, PHE 649, LEU 650, ILE 441, ASP 404, TRP 376, SER 676, LEU 677, LEU 678, ARG 600, ALA 555), 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon (ARG 600, ARG 672, HIS 674, SER 676, LEU 677, MET 519, ASP 518, TRP 516, ILE 441, ASP 404, LEU 405, TRP 376, TRP 481, PHE 649, LEU 650), Silane, [1-(5-hexenyl)-2methylenecyclopropy (LEU 650, PHE 649, SER 676, ASP 616, TRP 613, TRP 481, ASP 518, MET 519, LEU 405, TRP 376, ASP 282), Zingiberene (LEU 678, LEU 677, SER 676, HIS 674, ARG 672, LEU 650, PHE 649, TRP 613, GLY 615, ASP 616, ILE 441, TRP 516, TRP 376, ASP 518, MET 519, TRP 481), Cyclopentane, heneicosyl-(CAS) Heneicosane (THR 556, ALA 555, ALA 554,THR 551, GLY 549, ARG 527, ILE 526, PHE 525, ASN 524, SER 523, TRP 376, MET 519, ASP 518, TRP 516, TRP 613, PHE 649, HIS 674, ARG 672, ARG 600, ILE 441), 1,3-dioxolane, 2-(phenylmethyl)-(MET 519, ASP 518, LEU 405, ASP 404, TRP 376, HIS 674, SER 676, PHE 649, SER 523, ARG 600, PHE 525, ILE 441, TRP 481), Benzoldicarbonsaeure (GLY A:651, LEU A:650, PHE A: 649, LEU A:678, LEU A: 677, SER A: 676, ARG A: 411, TRP A: 481), dl-Citronellol (ASP 616, GLY 615, TRP 613, ILE 441, TRP 376, TRP 516, ASP 518, MET 519, PHE 525, TRP 516, ASP 518, MET 519, LEU 405, ASP 404, PHE 649, HIS 674), 9,12,15-Octadecatrienoic acid, methyl ester (LEU 405, ASP 404, TRP 376, HIS 674, SER 676, LEU 677, LEU 678, SER 523, PHE 525, TYR 292, ILE 441, TRP 516, ASP 518, MET 519, PHE 649, LEU 650, ALA 284), 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol (LEU 650, PHE 649, ASP 616, GLY 615, TRP 613, SER 676, HIS 674, ARG 600, ARG 672, LEU 405, TRP 481, TRP 516, ILE 441, TRP 376), 5,8,11,14-Icosatetraynoic Acid (TRP 618, ASP 616, LEU 283, ALA 284, LEU 650, PHE 649,

ARG 600, TRP 613, MET 519, ASP 518, TRP 516, SER 676, HIS 674, ARG 672, LEU 405, TRP 481, TRP 376, ILE 441), Nonadecnoic Acid (LEU 677, ARG 411, ASP 404, LEU 405, TRP 376, MET 519, TRP 516, TRP 613, PHE 649, ILE 441, LEU 677, TRP 481, PHE 525, SER 523), 1, E-11, Z-13-Octadecatriene (LEU 678, LEU 677, SER 676, HIS 674, ARG 672, PHE 649, LEU 650, GLY 615, MET 519, ASP 518, TRP 481, ILE 441, LEU 405), Docosane (GLY 483, TRP 481, VAL 480, LYS 479, SER 676, HIS 674, TRP 516, LEU 405, TRP 376, LEU 650, PHE 649, ALA 284, LEU 283, PHE 525), Hexadecanoic acid (CAS) Palmitic acid (LEU 678, LEU 677, SER 676, ARG 411, TRP 481, TRP 376, ILE 441, ASP 404, LEU 405, TRP 516, ARG 600, PHE 649, LEU 650), 9-Octadecenoic acid (Z)- (CAS) Oleic acid (ALA 284, LEU 283, ASP 282, ARG 281, ASP 616, GLY 615, TRP 613, HIS 674, ARG 672, TRP 376, TRP 516, ASP 518, MET 519, TRP 481, PHE 525, ILE 441), 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth (HIS A: 674, SER A:676. LEU A: 677, LEU A: 678, SER A: 679, TRP A: 618, GLY A: 615, TRP A: 613, MET A: 519, HIS A: 674, GLY A: 651, LEU A: 650, PHE A: 649, ILE A: 441, TRP A: 376, TRP A: 481), 14-Heptadecenal (GLY A: 651, LEU A: 650, PHE A: 649, LEU A: 678, SER A: 676, HIS A: 674, ARG A: 672, TRP A: 618, ASP A: 616, GLY A: 615, TRP A: 613, TRP A: 516, ILE A: 441, LEU A: 405, TRP A: 481, TRP A: 376), 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl (PHE 525, SER 523, MET 519, ASP 518, TRP 516, ASP 616, TRP 613, ARG 600, PHE 649, ILE 441, ASP 404, LEU 405, TRP 376, TRP 481), 1,2,3-Propanetriol, diacetate (CAS) Diacetin (TRP 613, ARG 600, GLY 615, ASP 616, MET 519, ASP 518, TRP 516, ILE 441, TRP 376, PHE 649, TRP 481, HIS 674, PHE 525, ASP 282), Propanoyl chloride (LEU 678, LEU 677, SER 676, PHE 649, TRP 376, TRP 481), Methane, Thiobis- (LEU 117, ALA 120, GLN 121, MET 122, GLY 123, GLN 124, PRO 125, TRP 126, CYS 127, ASP 91, CYS 92, ALA 93, PRO 94), n- Allyloxymethyl acrylamide (ARG 600, ASP 616, GLY 615, TRP 613, PHE

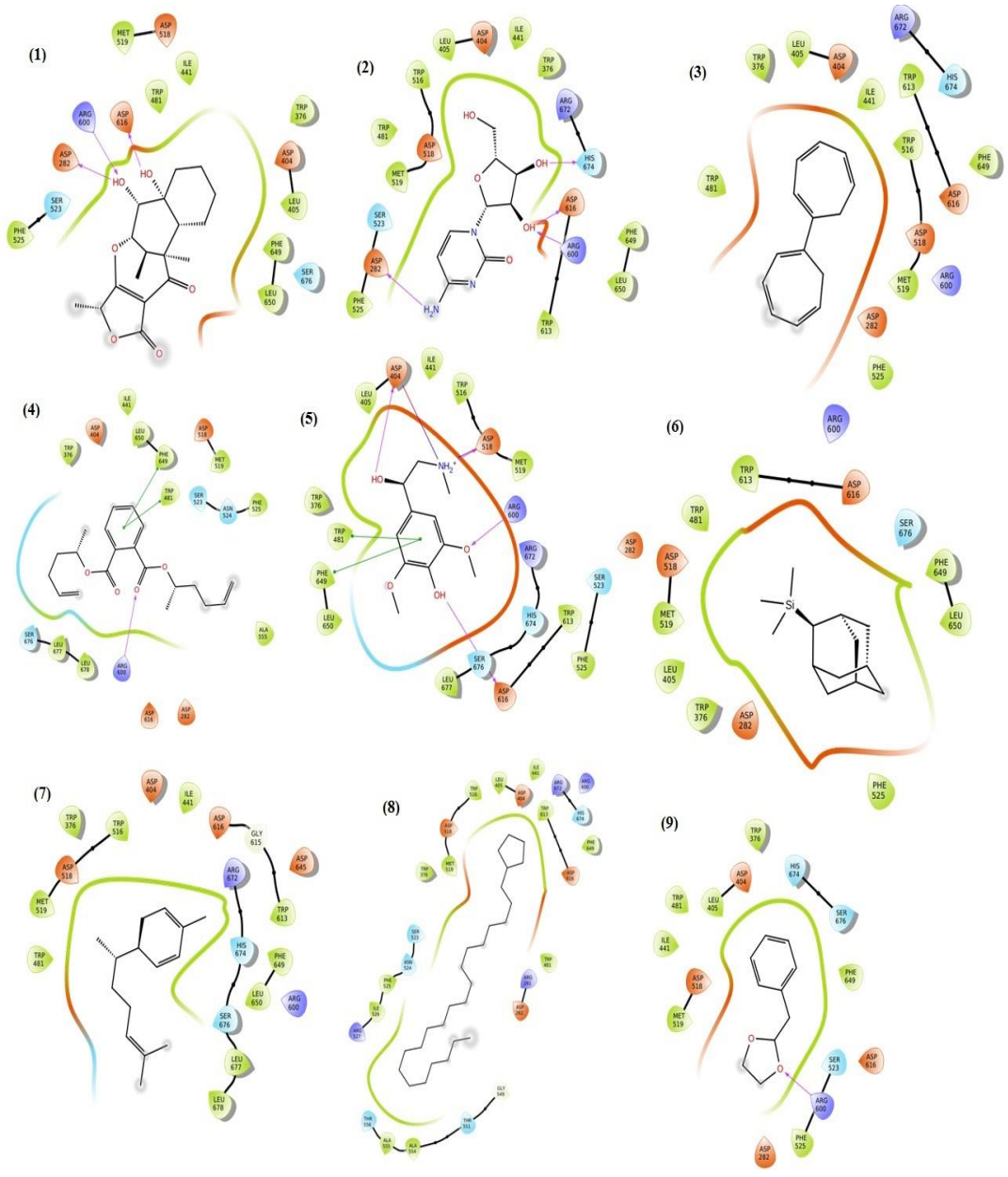
649, ASP 645, HIS 674, ARG 672, TRP 516, LEU 405, ILE 441, TRP 481, TRP 376). Dodecanoic acid (CAS) Lauric acid (GLY A: 651, LEU A: 650, PHE A649, TRP A: 376, MET A: 519, LEU A: 405, LEU A: 405, ASP A: 404, TRP A: 481, ILE A: 441, ARG A: 600, SER A: 679, LEU A: 678, LEU A: 677, SER A: 676), 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy (ARG A: 600, TRP A: 376, SER A: 676, LEU A: 677, LEU A: 678, SER A: 679, NAG F:1, GLY A: 651, LEU A: 650, PHE A: 649, LEU A: 405, ILE A: 441, TRP A: 481, MET A: 519, LEU A: 283 Trans-2-Phenyl-1,3-Dioxolane-4-M (ALA A: 284, ILE A: 526, PHE A: 525, ASN A: 524, SER A: 523, TRP A: 618, PHE A: 649, LEU A: 650, GLY A: 651, NAG F:1, SER A: 679, LEU A: 678, LEU A: 677, SER A: 676, TRP A: 376, ARG A: 600, MET A: 519, TRP A: 481), Acarbose (TRP 618, ASP 616, GLY 615, TRP 613, LEU 650, PHE 649, ARG 672, HIS 674, MET 519, TRP 481, TRP 516, LEU 405, ASP 404, ARG 600, ARG 281, ASP 282, LEU 283, PHE 525, SER 523, TRP 376, ILE 441, TRP 516, TRP 481, ASP 518, MET 519.

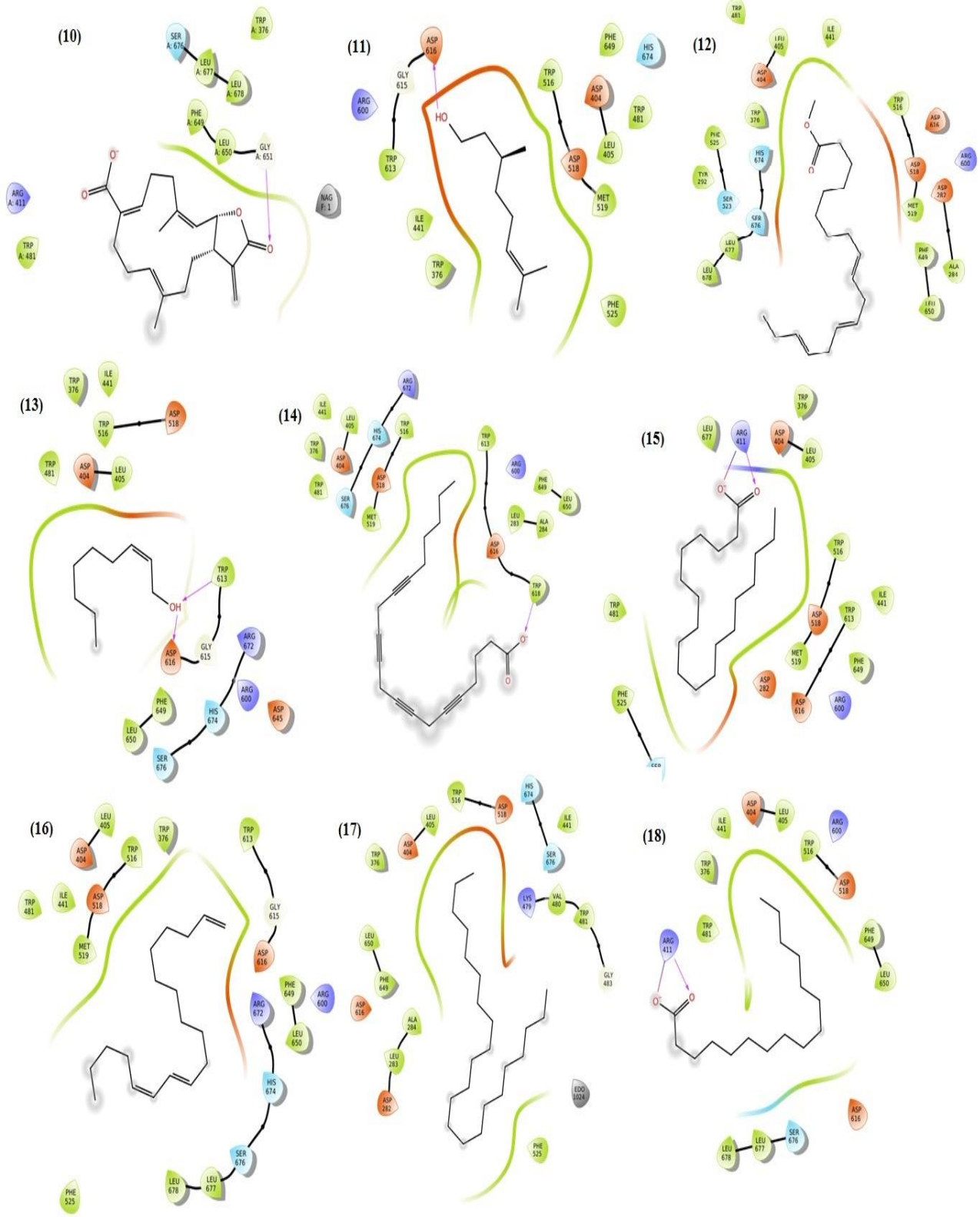
**TABLE 15: Docking Results of Bioactive Compounds from Leaves and Barks of *Pterocarpus indicus* in the Alpha Glucosidase Enzymes (PDB 2ZE0)**

| SI. NO | CID | Compound name | Glide score | Glide Evdw | Glide Ecoul | Glide Energy | Glide einternal | Glide emodel |
|---|---|---|---|---|---|---|---|---|
| 1 | 41774 | Acarbose | -14.452 | -34.354 | -40.022 | -74.377 | 12.268 | -110.596 |
| 2 | 31513 | 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | -7.496 | -22.241 | -17.619 | -39.861 | 2.708 | -55.803 |
| 3 | 5387599 | Trans-2-Phenyl-1,3-Dioxolane-4-M | -6.472 | -30.783 | -12.622 | -43.405 | 10.98 | -52.35 |
| 4 | 6175 | Cytidine (CAS) Cyd | -6.233 | -26.237 | -18.28 | -44.517 | 10.762 | -52.165 |
| 5 | 3074876 | Phthalic acid, butyl ester, ester with butyl glyc | -5.036 | -22.418 | -8.467 | -30.885 | 0.957 | -38.233 |
| 6 | 563003 | Bi-1,3,5-cycloheptatrien-1-yl (CAS) | -4.515 | -26.024 | -1.323 | -27.347 | 0.137 | -36.289 |
| 7 | 5358705 | Benzoldicarbonsaeure | -4.374 | -18.681 | -0.322 | -19.004 | 1.764 | -22.237 |
| 8 | 7057919 | 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | -4.253 | -37.551 | -3.12 | -40.671 | 4.37 | -56.734 |
| 9 | 59029 | Phthalic acid, di-(1-hexen-5-yl) ester | -3.72 | -33.718 | -2.671 | -36.389 | 3.806 | -49.522 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 10 | 92776 | Zingiberene | -3.655 | -19.428 | -0.916 | -20.344 | 6.193 | -20.156 |
| 11 | 8842 | dl-Citronellol | -3.604 | -12.975 | -10.727 | -23.701 | 2.041 | -28.447 |
| 12 | 7562 | 1,3-dioxolane, 2-(phenylmethyl)- | -3.575 | -19.13 | -3.929 | -23.059 | 0.077 | -28.778 |
| 13 | 5364941 | 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | -3.427 | -16.674 | -8.696 | -25.37 | 2.967 | -26.334 |
| 14 | 547946 | 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth | -3.338 | -27.335 | -6.123 | -33.458 | 9.274 | -44.019 |
| 15 | 66021 | 1,2,3-Propanetriol, diacetate (CAS) Diacetin | -3.295 | -13.903 | -14.271 | -28.175 | 4.532 | -33.228 |
| 16 | 62324 | Propanoyl chloride | -2.824 | -8.15 | -1.941 | -10.091 | 1.481 | -11.408 |
| 17 | 985 | Hexadecanoic acid (CAS) Palmitic acid | -2.734 | -19.031 | -6.493 | -25.523 | 3.072 | -29.573 |
| 18 | 13131343 | Silane, [1-(5-hexenyl)-2methylenecyclopropy | -2.404 | -16.347 | -0.134 | -16.481 | 0.033 | -15.498 |
| 19 | 12405 | Docosane | -2.39 | -25.241 | -0.433 | -25.674 | 0.965 | -34.457 |
| 20 | 12591 | Nonadecnoic Acid | -2.248 | -19.322 | -5.969 | -25.291 | 4.91 | -27.174 |
| 21 | 5362893 | 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl | -1.976 | -15.472 | -2.102 | -17.575 | 0.196 | -20.486 |
| 22 | 1780 | 5,8,11,14-Icosatetraynoic Acid | -1.974 | -22.296 | -2.929 | -25.225 | 1.126 | -31.137 |
| 23 | 3893 | Dodecanoic acid (CAS) Lauric acid | -1.367 | -16.276 | -1.5 | -17.776 | 2.462 | -19.47 |
| 24 | 1068 | Methane, Thiobis- | -1.048 | -10.182 | -1.872 | -12.054 | 0 | -12.852 |
| 25 | 5367667 | 14-Heptadecenal | -0.888 | -24.365 | -2.036 | -26.402 | 3.429 | -35.078 |
| 26 | 5367462 | 9,12,15-Octadecatrienoic acid, methyl ester | -0.651 | -25.994 | -3.476 | -29.47 | 3.336 | -35.759 |
| 27 | 5365585 | 1, E-11, Z-13-Octadecatriene | 0.091 | -21.473 | -0.858 | -22.331 | 0.978 | -30.004 |
| 28 | 445639 | 9-Octadecenoic acid (Z)- (CAS) Oleic acid | 0.291 | -23.106 | -0.491 | -23.597 | 3.643 | -29.258 |
| 29 | 23171 | Cyclopentane, heneicosyl- (CAS) Heneicosane | 0.919 | -32.946 | -0.156 | -33.102 | 1.378 | -45.847 |
| 30 | 19357475 | n- Allyloxymethyl acrylamide | 1.222 | -18.764 | -4.482 | -23.247 | 2.204 | -28.255 |

**Figure 15: Molecular docking analysis of 1)Phthalic acid, butyl ester, ester with butyl glyc 2) Cytidine (CAS) Cyd 3) Bi-1,3,5-cycloheptatrien-1-yl (CAS) 4) Phthalic acid, di-(1-hexen-5-yl) ester 5) 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon 6) Silane, [1-(5-hexenyl)-2methylenecyclopropy 7) Zingiberene 8) Cyclopentane, heneicosyl- (CAS) Heneicosane 9) 1,3-dioxolane, 2-(phenylmethyl)-10) Benzoldicarbonsaeure 11) dl-Citronellol 12) 9,12,15-Octadecatrienoic acid, methyl ester 13) 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol 14) 5,8,11,14-Icosatetraynoic Acid 15) Nonadecnoic Acid 16) 1, E-11, Z-13-Octadecatriene 17) Docosane 18) Hexadecanoic acid (CAS) Palmitic acid 19) 9-Octadecenoic acid (Z)- (CAS) Oleic acid 20) 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth 21) 14-Heptadecenal 22) 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl 23) 1,2,3-Propanetriol, diacetate (CAS) Diacetin 24) Propanoyl chloride 25) Methane, Thiobis- 26) n- Allyloxymethyl acrylamide 27) Dodecanoic acid (CAS) Lauric acid 28) 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy 29) Trans-2-Phenyl-1,3-Dioxolane-4-M 30) Acarbose with alpha amylase glucosidase (pdb 2ze0) obtained from GLIDE docking.**

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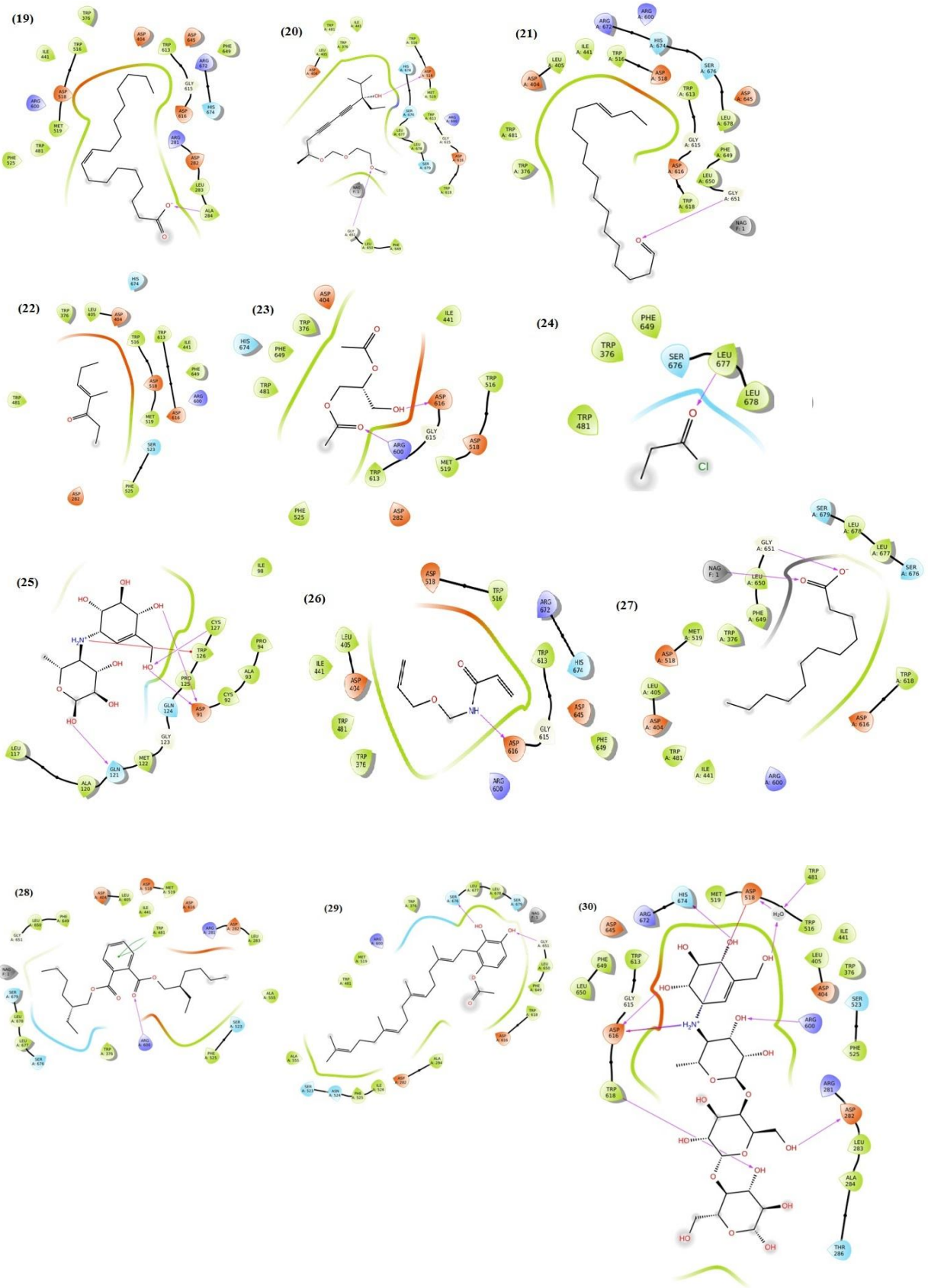
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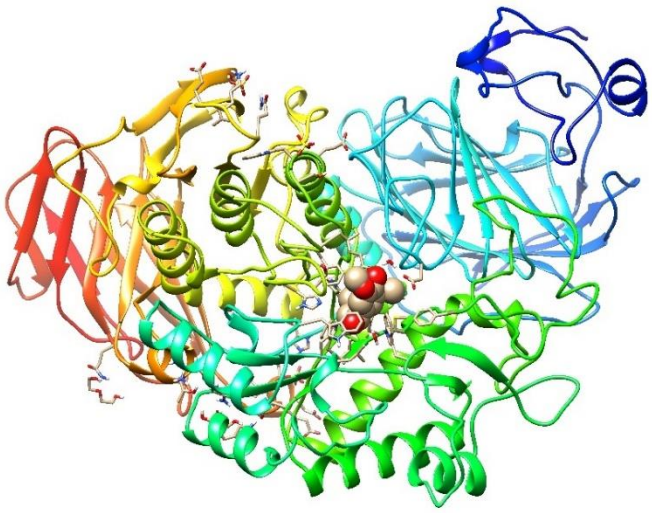


**FIGURE:16 Compound 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon Docked with the Binding Site of Alpha Glucosidase**

## 4.9 *In Silico* Docking Analysis of Phytochemicals Compounds from the Barks and Leaves of *Pterocarpus indicus* for Estrogen Receptor Alpha (ER-α)

Grid based docking study was used to analysis the binding modes of molecules with the amino acids present in the active side of the protein in order to study the interaction of the compounds with the estrogen receptor alpha (ER-α) (Figure 17). We also performed glide docking analysis by Schrodinger, where compared to all the compounds phthalic acid, butyl ester, ester with butyl glyc (-8.827) followed by cytidine (CAS) cyd (-8.781), Bi-1,3-5-cyclo heptatrien-1-yl (CAS) (-8.353) showed the best docking score as shown in (Table 16) due to the strong binding bond between 3 ERT and compounds in favourable conformation. Tamoxifen (-12.982) is an antagonist of ER-alpha used to control the breast cancer and also it binds the amino acids and blocks the function of estrogen receptor and inhibits the function of human estrogen receptor. The results of the docking analysis were 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl :ARG:394, LEU:391, MET:388, LEU:387,LEU:384, ALA:350, LEU:349, LEU:346,

PHE:404, MET:421, ILE:424, LEU:428 1,2,3-Propanetriol, diacetate (CAS) Diacetin : ALA:350, LEU:349, THR:347, LEU:346, LEU:525, MET:343, TRP:383, LEU:384, LEU:387, MET:388, LEU:391, ARG:394, PHE:404 , ILE:424, MET:421, LEU:428. 1,2,3-Propanetriol, diacetate (CAS) Diacetin : LEU:387, LEU:384, PHE:404, ALA:350, LEU:349, THR:347, LEU:346, MET:343, VAL:481, GLY:420, MET:421, ILE:424, ARG:394, LEU:391, MET:388, LEU:428, LEU:525, HIE:524, GLY:52149, Zingiberene: ALA:350, LEU:349, LEU:346, MET:343, ARG:394, LEU:391, MET:388, LEU:387, LEU:384, PHE:404, VAL:418, GLY:420, MET:421, ILE:424, GLY:521, HIE:524, LEU:525, LEU:428. Hexadecanoic acid (CAS)Palmitic acid: ARG:394, LEU:391, MET:388, LEU:387, LEU:428, LEU:384, TRP:383, GLY:420, MET:421, ILE:424, GLY:521, HIE:524, LEU:525, MET:434, LEU:346, THR:347, LEU:349, ALA:350, PHE:404. Benzoldicarbonsaeure: ARG:394, PHE:404, LEU:391, MET:388, LEU:387, LEU:384, TRP:383, GLU:353, ALA:350, LEU:349, THR:347, LEU:346, MET:343, VAL:418, GLU:419, GLY:420, MET:421, ILE:424, LEU:525, HIE:524, GLY:521, LEU:428. Dodecanoic acid (CAS) Lauric acid: ARG:394, LEU:391, MET:388, PHE:404, LEU:387, LEU:384, LEU:428, GLY:521, HIE:524, LEU:525, ILE:424, MET:421, GLY:420, MET:343, LEU:346, LEU:349, ALA:350. Cyclopentane, heneicosyl- (CAS) Heneicosane: LEU:391, MET:388, LEU:387, LEU:384, TRP:383, ILE:424, MET:421, LEU:428, PHE: 404, MET:343, LEU:346, THR:347, ALA:350, LEU:536, TYR:526, LEU:525, MET:522. 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol: ARG:394, LEU:391, MET:388, LEU:387, LEU:384, PHE:404, ILE:424, LEU:428, MET:421, GLY:420, VAL:418, ALA:350, LEU:349, LEU:346, MET:343, GLY:521, HIE:524, LEU:525. 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon: ALA:350, LEU:349, THR: 347, LEU:346, MET:343, PHE:404, LEU:384, LEU:387, MET:388, LEU:391, ARG:394, LEU:525, HIE:524, MET:421, ILE:424, LEU:428. Nonadecnoic

Acid: CYS:530, VAL:533, LYS:529, MET:528, LEU:525, ILE:424, MET:421, GLY:521, MET:343, LEU:346, THR:347, LEU:349, ALA:350, TRP:383, LEU:384, LEU:428, LEU:387, MET:388, PHE:404, LEU:392, ARG:394. Phthalic acid, butyl ester, ester with butyl glyc: LEU:525, TYR:526, MET:528, LYS:529, CYS:530, VAL:533, LEU:536, MET:522, THR:347, ALA:350, TRP:383. Bi-1,3,5-cycloheptatrien-1-yl (CAS): LEU:384, LEU:387, MET:388, LEU:391, ARG:394, ALA:350, LEU:349, LEU:346, MET:343, PHE:404, VAL:418, GLY:420, MET:421, ILE:424, GLY:521, HIE:524, LEU:525. 5,8,11,14-Icosatetraynoic Acid: CYS:530, LYS:529, VAL:533, LEU:536, PHE:404, LEU:428, ILE:424, MET:421, LEU:391, MET:388, LEU:387, LEU:384, TRP:383, LEU:525, MET:343, LEU:346, THR:347, ALA:350, LEU:354. dl-Citronellol: ARG:394, LEU:391, MET:388, LEU:387, LEU:384, TRP:383, ALA:350, LEU:349, LEU:428, LEU:346, MET:343, MET:421, ILE:424, PHE:404, LEU:525. 1, E-11, Z-13-Octadecatriene: TRP:383, LEU:384, LEU:387, MET:388, LEU:428, LEU:391, ARG:394, PHE:404, ALA:350, LEU:349, THR:347, LEU:346, MET:434, ILE:424, MET:421, MET:528, LEU:525, HIE:524, GLY:521. 9,12,15-Octadecatrienoic acid, methyl ester: ARG:394, PHE:404, LEU:391, MET:388, LEU:387, LEU:384, TRP:383, MET:421, ILE:424, LEU:428, LEU:536, LEU:354, ALA:350, LEU:349, LEU:525, THR:347, LEU:346, MET:343. 9-Octadecenoic acid (Z)- (CAS) Oleic acid: CYS:530, VAL:533, LYS:529, MET:528: TYR:526, LEU:525, ALA:350, THR:347, LEU:346, MET:343, MET:421, ILE:424, LEU:391, PHE:404, LEU:428, MET:388, LEU:387, LEU:384, TRP:383. Trans-2-Phenyl-1,3-Dioxolane-4-M: ARG:394, LEU:391, LEU:428, PHE:404, MET:388, LEU:387, LEU:384, LEU:383, ILE:424, MET:421, LEU:525, TYR:526, MET:528, LYS:529, CYS:530, VAL:533, PRO:525, MET:343, LEU:346, THR:347, LEU:349, ALA:350. 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy: CYS:530, LYS:529, MET:528, TYR:526, LEU:525, MET:522, MET:343,

LEU:346, THR:347, ALA:350, LEU:387, LEU:354, TRP:383, VAL:533, VAL: 534, PRO:535, LEU:536, LEU:539. Docosane: ILE:424, MET:421, MET:343, LEU:346, THR:347, ALA:350, LEU:536, VAL: 533, CYS:530, LYS:529, MET:528, TYR:526, LEU:525, MET:522, GLY:521, PHE:404, LEU:391, LEU:428, MET:388, LEU:387, LEU:384, TRP:383. Phthalic acid, di-(1-hexen-5-yl) ester: TRP:383, LEU:384, LEU:387, MET:388, LEU:428, LEU:391, PHE:404, ARG:394, ALA:350, LEU:349, THR:347, LEU:346, MET:343, VAL:418, GLY:420, MET:421, ILE:424, LEU:525, HIE:524, GLY:521. Propanoyl chloride: ARG:394, LEU:391, MET:388, LEU:387, LEU:384, LEU:346, LEU:349, ALA:350, PHE:404. 1,3-dioxolane,2-(phenylmethyl)-: LEU:391, LEU:428, MET:388, LEU:387, LEU:384, LEU:346, PHE:404, MET:343, VAL:418, GLY:420, MET:421, ILE:424, LEU:525, HIE:524, GLY: 521.n-Allyloxymethyl acrylamide: LEU:384, LEU:387, MET:388, LEU:391, LEU:428, GLY:521, HIE:524, LEU:525, GLY:420, MET:421, ILE:424, PHE:404, LEU:346, MET:343. Silane, [1-(5-hexenyl)-2methylenecyclopropy: ILE:424, MET:421, GLY:420, VAL:418, LEU:428, PHE:404, LEU:391, MET:388, LEU:387, ALA:350, LEU:384, TRP:383, LEU:525, HIE:524, GLY:521, MET:343, LEU:346. 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth: LEU:354, ALA:350, LEU:536, THR:347, LEU:346, PHE:404, MET:343, GLY:420, MET:421, ILE:424, LEU:391, MET:388, LEU:387, LEU:384, TRP:383, LEU:525, HIE:524, MET:522, GLY:521, LEU:428. Methane, Thiobis- (LEU A: 385, LEU A: 387, LEU A: 391, MET A: 421, LEU A: 428, MET A: 388, PHE A: 404, ILE A: 424. 14-Heptadecenal MET 343, LEU 346, THR 347, LEU 349, ALA 350, ASP 351, GLU 353, ARG 394, LEU 391, MET 388, LEU 387, LEU 384, TRP 383, GLY 521 Tamoxifen: LEU:354, ALA:350, LEU:349, LEU:536, THR:347, LEU:346, MET:343, PHE:404, ARG:394, LEU:428, LEU:391, MET:388, LEU:387,

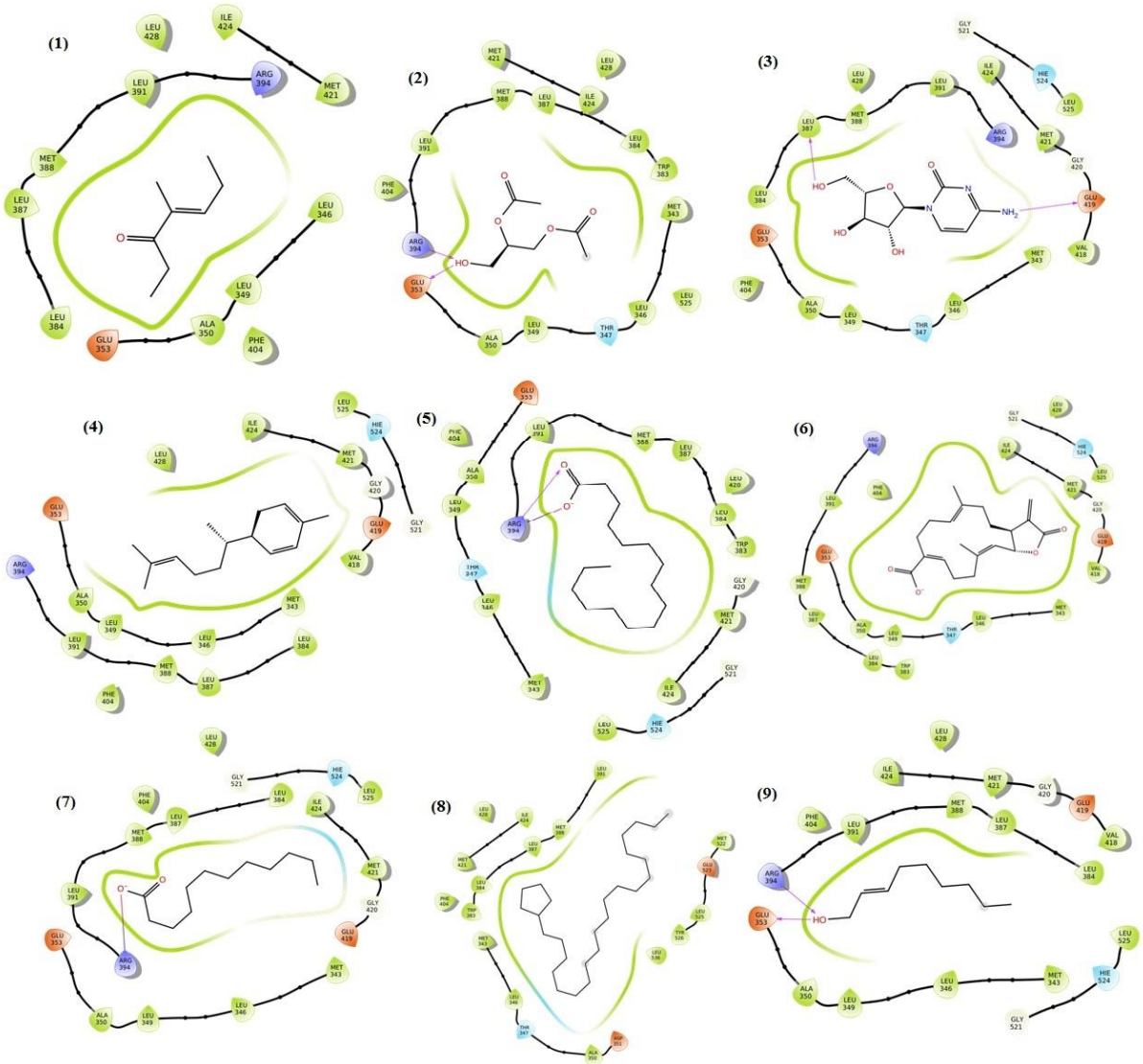
LEU:384, TRP:383, VAL:418, GLY:420, MET:421, ILE:424, LEU:525, HIE:524, GLY:521.

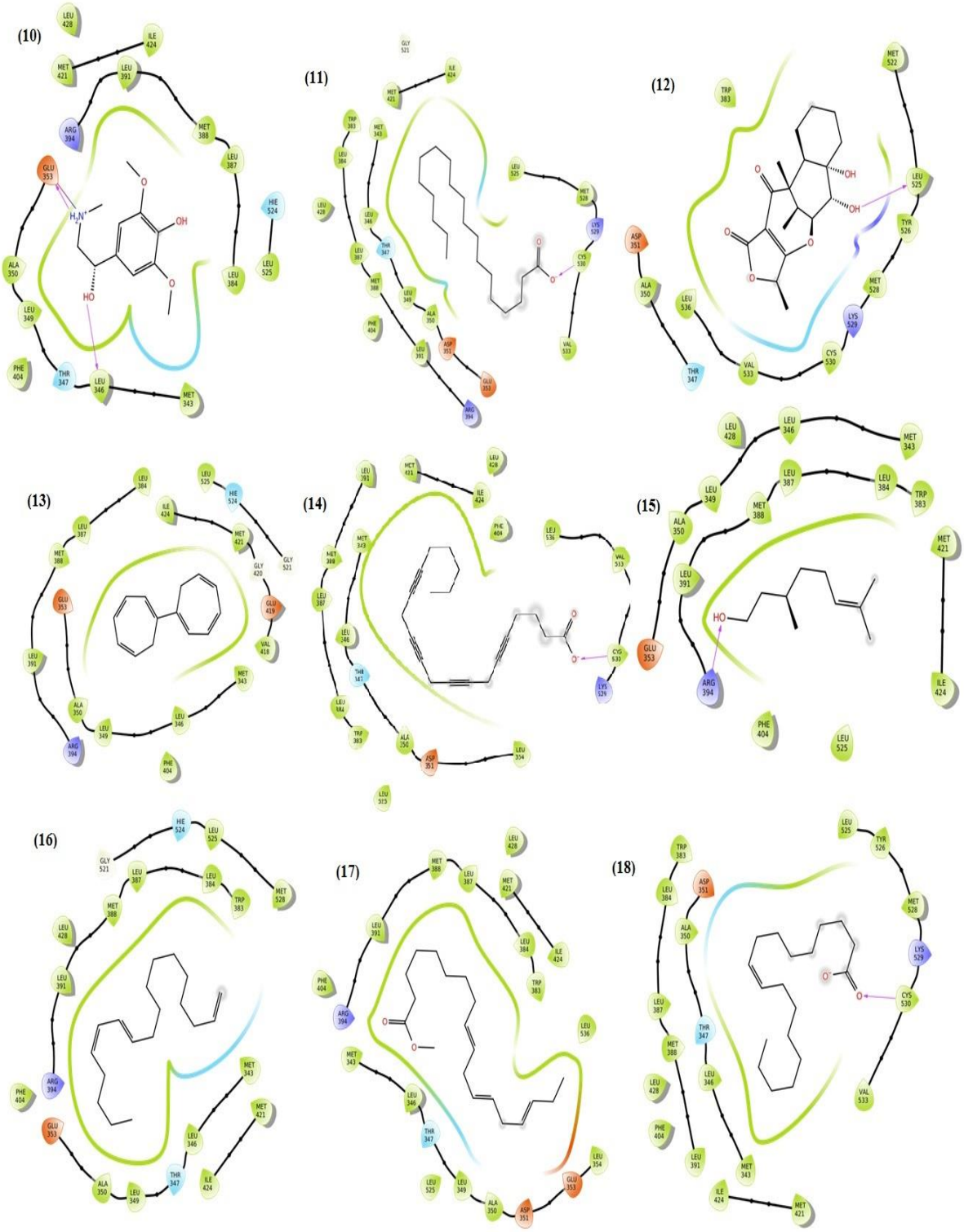
**TABLE 16: Docking Results of Bioactive Compounds from Leaves and Barks of *Pterocarpus indicus* for Estrogen Receptor Alpha (ER-α) (PDB: 3ERT)**

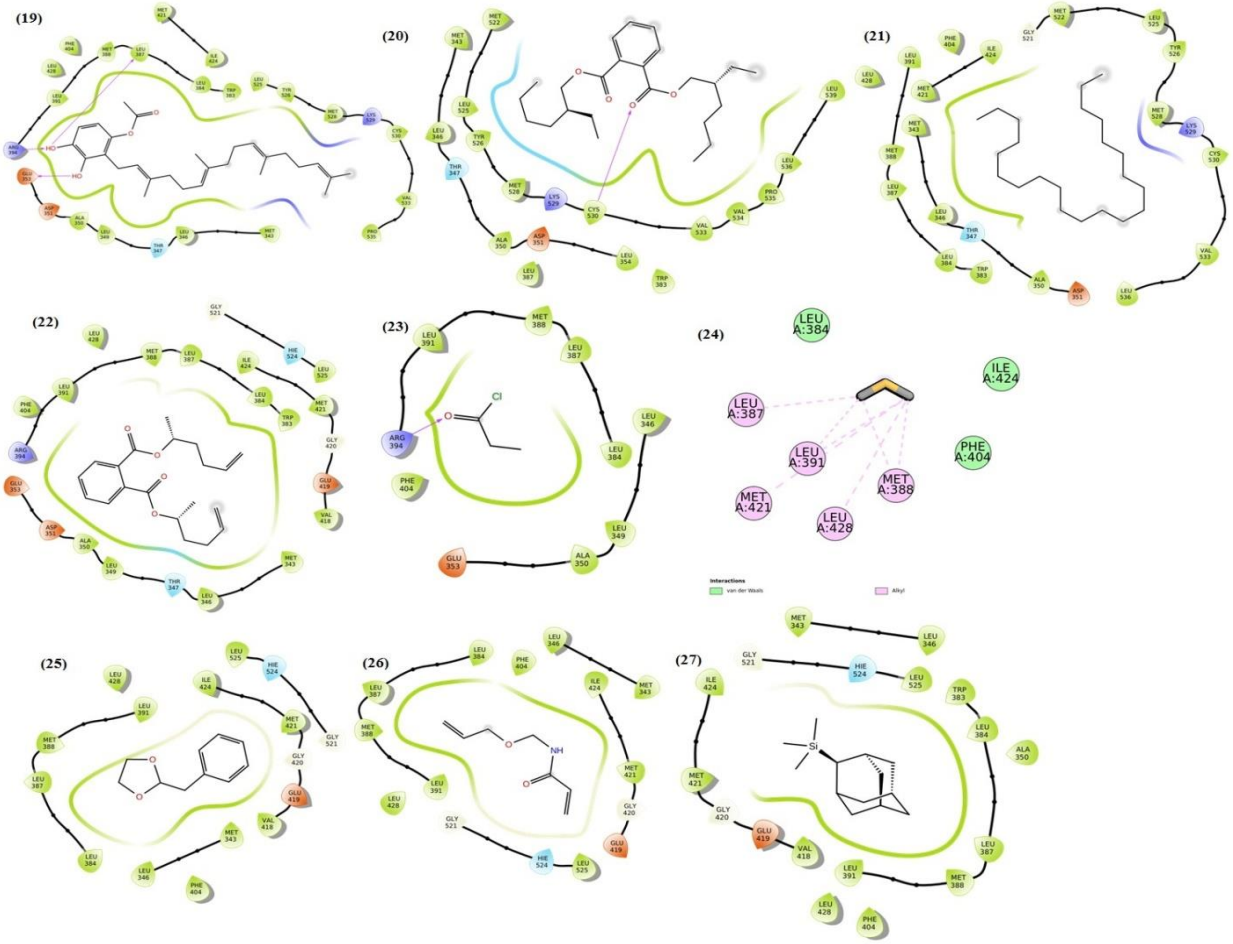
| S. No | CID | Compound Name | Glide score | Glide Evdw | Glide Ecoul | Glide Energy | Glide einternal | Glide emodel |
|---|---|---|---|---|---|---|---|---|
| 1. | 2733526 | Tamoxifen | -12.982 | -50.584 | -4.877 | -55.561 | 6.365 | -88.554 |
| 2. | 5387599 | Trans-2-Phenyl-1,3-Dioxolane-4-M | -9.721 | -44.105 | -5.193 | -49.299 | 5.699 | -88.554 |
| 3. | 3074876 | Phthalic acid, butyl ester, ester with butyl glyc | - 8.827 | -18.788 | -2.78 | -21.578 | 0.329 | -16.442 |
| 4. | 6175 | Cytidine (CAS) Cyd | -8.781 | -24.470 | -4.473 | -28.944 | 3.856 | -41.093 |
| 5. | 563003 | Bi-1,3,5-cycloheptatrien-1-yl (CAS) | -8.353 | -29.879 | -0.246 | -30.125 | 0.450 | -39.486 |
| 6. | 59029 | Phthalic acid, di-(1-hexen-5-yl) ester | -8.176 | -42.255 | -1.781 | -44.637 | 10.578 | -58.378 |
| 7. | 31513 | 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | -8.039 | -27.404 | -6.247 | -33.651 | 1.910 | -44.753 |
| 8. | 13131343 | Silane, [1-(5-hexenyl)-2methylenecyclopropy | -7.889 | -24.404 | -0.567 | -23.484 | 5.860 | -29.009 |
| 9. | 92776 | Zingiberene | -7.719 | -25.462 | -0.167 | -25.629 | 0.687 | -32.203 |
| 10. | 23171 | Cyclopentane, heneicosyl- (CAS) Heneicosane | -6.923 | -31.534 | -0.003 | -31.537 | 3.030 | -42.452 |
| 11. | 5358705 | Benzoldicarbonsaeure | -6.917 | -13.917 | 1.086 | -12.110 | 2.575 | -54.612 |
| 12. | 7562 | 1,3-dioxolane,2-(phenylmethyl)- | -6.411 | -21.241 | 0.288 | -20.953 | 0.458 | -26.711 |
| 13. | 5367462 | 9,12,15-Octadecatrienoic acid, methyl ester | -6.250 | -39.174 | -1.146 | -40.590 | 7.818 | -46.161 |
| 14. | 8842 | dl-Citronellol | -5.953 | -17.124 | -5.299 | -22.422 | 2.214 | -27.613 |

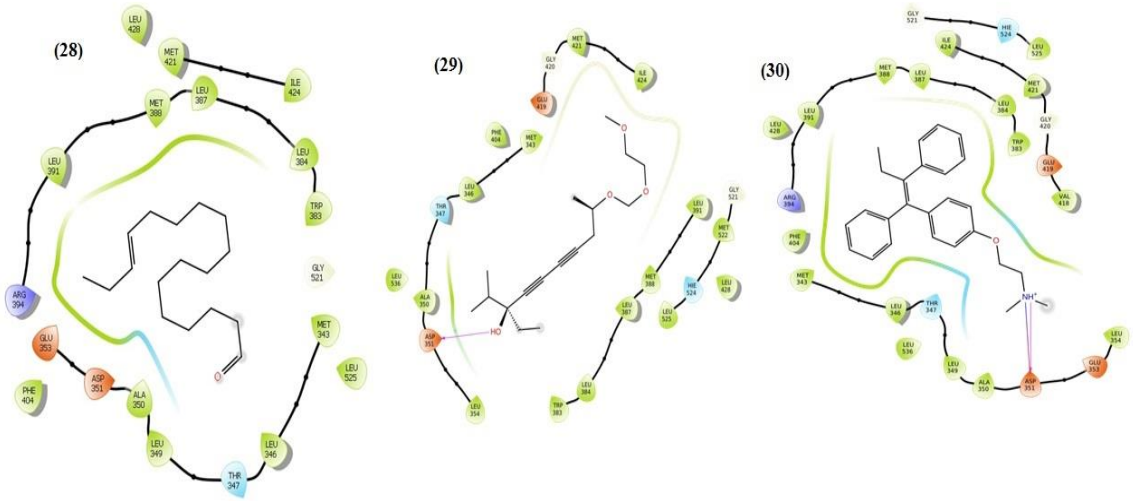
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15. | 7057919 | 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | -5.891 | -34.098 | -3.410 | -37.238 | 5.886 | -47.643 |
| 16. | 5364941 | 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | -5.768 | -18.716 | -5.271 | -23.987 | 3.313 | -28.772 |
| 17. | 1780 | 5,8,11,14-Icosatetraynoic Acid | -5.526 | -29.718 | -3.211 | -32.929 | 5.752 | -40.479 |
| 18. | 5365585 | 1, E-11, Z-13-Octadecatriene | -5.511 | -29.932 | -0.148 | -30.080 | 5.067 | -38.293 |
| 19. | 12591 | Nonadecnoic Acid | -5.221 | -27.441 | -2.887 | -30.327 | -4.529 | -33.471 |
| 20. | 12405 | Docosane | -5.123 | -28.187 | 0.107 | -28.080 | 1.822 | -38.392 |
| 21. | 985 | Hexadecanoic acid (CAS) Palmitic acid | -4.842 | -26.979 | 0.316 | -26.663 | 1.944 | -33.633 |
| 22. | 445639 | 9-Octadecenoic acid (Z)- (CAS) Oleic acid | -4.810 | -25.212 | -2.690 | -27.902 | 5.371 | -32.397 |
| 23. | 547946 | 4,6-Decadiyn-3-ol, 3-isopropyl-9-(methoxyeth | -4.751 | -32.722 | -5.769 | -38.941 | 7.101 | -46.639 |
| 24. | 5367667 | 14-Heptadecenal | -4.445 | -31.668 | -0.585 | -32.253 | 4.569 | -38.798 |
| 25. | 5362893 | 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl | -4.094 | -17.387 | 0.479 | -16.908 | 2.795 | -18.689 |
| 26. | 66021 | 1,2,3-Propanetriol, diacetate (CAS) Diacetin | -3.857 | -19.919 | -8.735 | -28.654 | 3.462 | -34.414 |
| 27. | 3893 | Dodecanoic acid (CAS) Lauric acid | -3.838 | -23.118 | 0.228 | -22.889 | 2.734 | -24.134 |
| 28. | 62324 | Propanoyl chloride | -1.718 | -14.929 | -0.445 | -15.374 | 1.475 | -14.606 |
| 29. | 1068 | Methane, Thiobis- | -1.557 | -9.858 | 0.236 | -9.633 | 0.000 | -10.932 |
| 30. | 19357475 | n- Allyloxymethyl acrylamide | 0.030 | -20.133 | -0.536 | -20.669 | 2.738 | -24.567 |

**Figure 17: Molecular docking analysis of 1)4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl 2) 1,2,3-Propanetriol, diacetate (CAS) Diacetin 3) Cytidine (CAS) Cyd 4) Zingiberene 5) Hexadecanoic acid (CAS)Palmitic acid 6) Benzoldicarbonsaeure 7) Dodecanoic acid (CAS) Lauric acid 8) Cyclopentane, heneicosyl- (CAS) Heneicosane 9) 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol 10) 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon 11) Nonadecnoic Acid 12) Phthalic acid, butyl ester, ester with butyl glyc 13) Bi-1,3,5-cycloheptatrien-1-yl (CAS) 14) 5,8,11,14-Icosatetraynoic Acid 15) dl-Citronellol 16) 1, E-11, Z-13-Octadecatriene 17) 9,12,15-Octadecatrienoic acid, methyl ester 18) 9-Octadecenoic acid (Z)- (CAS) Oleic acid 19) Trans-2-Phenyl-1,3-Dioxolane-4-M 20) 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy 21) Docosane 22) Phthalic acid, di-(1-hexen-5-yl) ester 23) Propanoyl chloride 24) Methane, Thiobis- 25) 1,3-dioxolane,2-(phenylmethyl)- 26) n- Allyloxymethyl acrylamide 27) Silane, [1-(5-hexenyl)-2methylenecyclopropy 28) 14-Heptadecena 29) 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth 30) Tamoxifen with human estrogen receptor (PDB: 3ERT) obtained from GLIDE docking.**




(10)
(11)
(12)
(13)
(14)
(15)
(16)
(17)
(18)


(19)
(20)
(21)
(22)
(23)
(24)
LEU A:384
ILE A:424
LEU A:387
LEU A:391
PHE A:404
MET A:388
MET A:421
LEU A:428
(25)
(26)
(27)


(28)
(29)
(30)

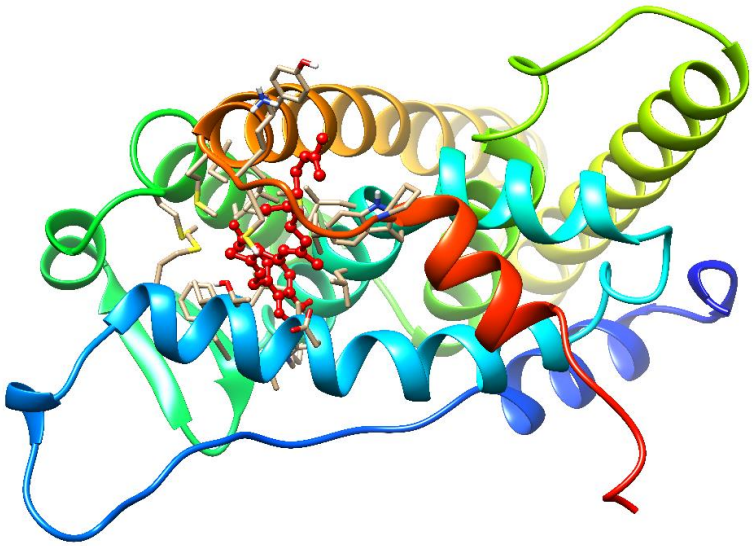


**FIGURE: 18 Compound Trans-2-Phenyl-1,3-Dioxolane-4-M Docked with the Binding Site of Human Estrogen Receptor Alpha**

### 4.10 Analysis of ADME Characteristics of Ligands

GI absorption, Skin permeability, substrate or inhibitor of P- glycoprotein was used to measure the absorption characteristics of drugs. The drug distribution depends on blood brain barrier (BBB). Cytochrome P450 model used for substrate CYP2D6 and CYP3A4, inhibitor CYP1A2, CYP2C19, CYP2C9 are used to determine the metabolism of drug. The total clearance model was used to depict the excretion of drug. Compounds like palmitic acid, Nanodecanoic acid, Oleic acid and 5-8-11-14-Eicosatetrayonic acid satisfy all ADME properties (Figure 19) like GI absorption, skin permeability, blood brain barrier etc. which clearly depict this as a potent drug. (Table 17).

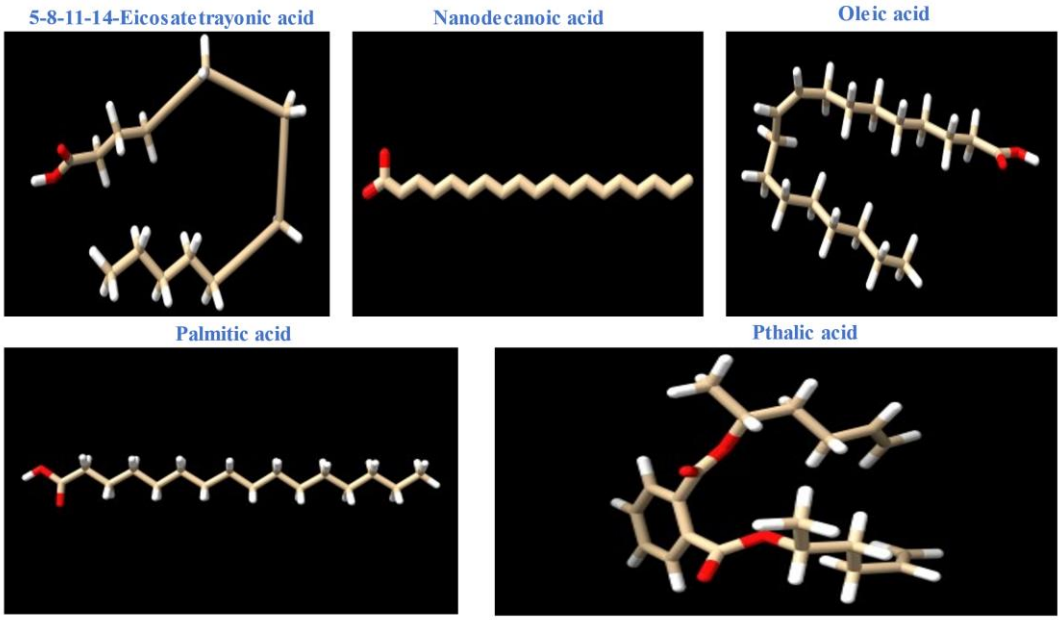



**FIGURE 19: Structure of Compounds Satisfy ADME Properties**

## 4.11 HOMO – LUMO Prediction

The energy difference between the Homo and LUMO is a HOMO – LUMO Gap, which reflect the chemical activity of the molecule. HOMO is the highest occupied molecular orbital and LUMO is the lowest unoccupied molecular orbital. Compounds like Benzoldicarbonsaeure (-0.07166), 9-Octadecenoic acid (Z)- (CAS) Oleic acid (-0.09856), Hexadecanoic acid (CAS) Palmitic acid (-0.12114), Dodecanoic acid (CAS) Lauric acid (-0.14372) shows lowest HLG (eV) value, which depict that these compounds may be a potent drug (Table 18).

## Table 17: Analysis of ADME Properties

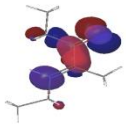
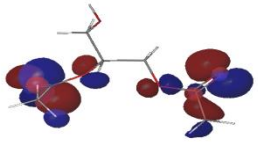
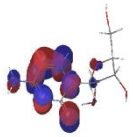
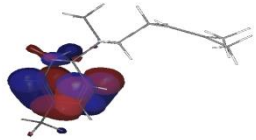
| Compound Name | Canonical SMILES | Formula | MW | #Heavy atoms | #Aromatic heavy atoms | Fraction Csp3 | #Rotatable bonds | #H-bond acceptors | #H-bond donors | MR | TPSA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Hepten-3-one,4-methyl- (CAS) 4-Methyl | CCC=C(C(=O)CC)C | $C_8H_{14}O$ | 126.2 | 9 | 0 | 0.62 | 3 | 1 | 0 | 40.3 | 17.07 |
| 1,2,3-Propanetriol, diacetate(CAS) Diacetin | OCC(OC(=O)C)COC(=O)C | $C_7H_{12}O_5$ | 176.17 | 12 | 0 | 0.71 | 6 | 5 | 1 | 39.49 | 72.83 |
| Cytidine (CAS) Cyd | OCC1OC(C(C1O)O)n1ccc(nc1=O)N | $C_9H_{13}N_3O_5$ | 243.22 | 17 | 6 | 0.56 | 2 | 6 | 4 | 55.85 | 130.83 |
| Zingiberene | CC(=CCCC(C1CC=C(C=C1)C)C)C | $C_{15}H_{24}$ | 204.35 | 15 | 0 | 0.6 | 4 | 0 | 0 | 70.68 | 0 |
| Hexadecanoicacid (CAS) Palmitic acid | CCCCCCCCCCCCCCCC(=O)O | $C_{16}H_{32}O_2$ | 256.42 | 18 | 0 | 0.94 | 14 | 2 | 1 | 80.8 | 37.3 |
| Benzoldicarbonsaeure | CC1=CCCC(=CCCC(=CC2C(CC1)C(=C)C(=O)O2)C)C(=O)O | $C_{20}H_{26}O_4$ | 330.42 | 24 | 0 | 0.5 | 1 | 4 | 1 | 95.19 | 63.6 |
| Dodecanoic acid (CAS) Lauric acid | CCCCCCCCCCCC(=O)O | $C_{12}H_{24}O_2$ | 200.32 | 14 | 0 | 0.92 | 10 | 2 | 1 | 61.57 | 37.3 |
| Cyclopentane, heneicosyl- (CAS) Heneicosane | CCCCCCCCCCCCCCCCCCCCCCC1CCCC1 | $C_{26}H_{52}$ | 364.69 | 26 | 0 | 1 | 20 | 0 | 0 | 124.98 | 0 |
| 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | CCCCCCC=CCO | $C_9H_{18}O$ | 142.24 | 10 | 0 | 0.78 | 6 | 1 | 1 | 46.06 | 20.23 |
| 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | CNCC(c1cc(OC)c(c(c1)OC)O)O | $C_{11}H_{17}NO_4$ | 227.26 | 16 | 6 | 0.45 | 5 | 5 | 3 | 59.99 | 70.95 |
| Nonadecanoic Acid | CCCCCCCCCCCCCCCCCCC(=O)O | $C_{19}H_{38}O_2$ | 298.5 | 21 | 0 | 0.95 | 17 | 2 | 1 | 95.22 | 37.3 |
| Phthalic acid, butyl ester, ester with butyl glyc | CC1OC(=O)C2=C1OC1C(C(C2=O)(C)C2C(C1O)(O)CCCC2)C | $C_{18}H_{24}O_6$ | 336.38 | 24 | 0 | 0.78 | 0 | 6 | 2 | 84.38 | 93.06 |
| Bi-1,3,5-cycloheptatrien-1-yl (CAS) | C1=CCC(=CC=C1)C1=CC=CC=CC1 | $C_{14}H_{14}$ | 182.26 | 14 | 0 | 0.14 | 1 | 0 | 0 | 62.34 | 0 |

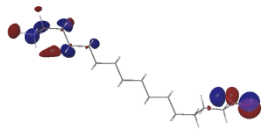
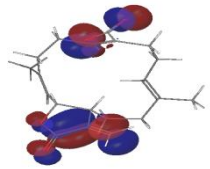
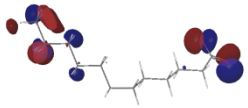
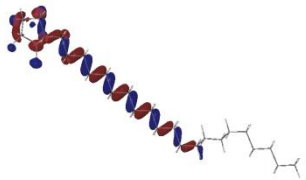
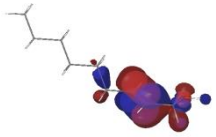
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5,8,11,14-Icosatetraynoic Acid | CCCCCC#CCC#CCC#CCC#CCCCC(=O)O | $C_{20}H_{24}O_2$ | 296.4 | 22 | 0 | 0.55 | 6 | 2 | 1 | 92.67 | 37.3 |
| dl-Citronellol | OCCC(CCC=C(C)C)C | $C_{10}H_{20}O$ | 156.27 | 11 | 0 | 0.8 | 5 | 1 | 1 | 50.87 | 20.23 |
| 1, E-11, Z-13-Octadecatriene | CCCCC=CC=CCCCCCCCCC=C | $C_{18}H_{32}$ | 248.45 | 18 | 0 | 0.67 | 13 | 0 | 0 | 87.22 | 0 |
| 9,12,15-Octadecatrienoic acid, methyl ester | CCC=CCC=CCC=CCCCCCCCC(=O)OC | $C_{19}H_{32}O_2$ | 292.46 | 21 | 0 | 0.63 | 14 | 2 | 0 | 93.31 | 26.3 |
| 9-Octadecenoic acid (Z)- (CAS) Oleic acid | CCCCCCCCC=CCCCCCCCC(=O)O | $C_{18}H_{34}O_2$ | 282.46 | 20 | 0 | 0.83 | 15 | 2 | 1 | 89.94 | 37.3 |
| Trans-2-Phenyl-1,3-Dioxolane-4-M | CC(=CCCC(=CCc1c(ccc(c1O)O)OC(=O)C)C)CCC=C(CCC=C(C)C)C | $C_{28}H_{40}O_4$ | 440.61 | 32 | 6 | 0.46 | 13 | 4 | 2 | 136.39 | 66.76 |
| 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | CCCCC(COC(=O)c1ccccc1C(=O)OCC(CCCC)CC)CC | $C_{24}H_{38}O_4$ | 390.56 | 28 | 6 | 0.67 | 16 | 4 | 0 | 116.3 | 52.6 |
| Docosane | CCCCCCCCCCCCCCCCCCCCCC | $C_{22}H_{46}$ | 310.6 | 22 | 0 | 1 | 19 | 0 | 0 | 107.87 | 0 |
| Phthalic acid, di-(1-hexen-5-yl) ester | C=CCCC(OC(=O)c1ccccc1C(=O)OC(CCC=C)C)C | $C_{20}H_{26}O_4$ | 330.42 | 24 | 6 | 0.4 | 12 | 4 | 0 | 96.12 | 52.6 |
| Propanoyl chloride | CCC(=O)Cl | $C_3H_5ClO$ | 92.52 | 5 | 0 | 0.67 | 1 | 1 | 0 | 21.53 | 17.07 |
| Methane, Thiobis- | CSC | $C_2H_6S$ | 62.13 | 3 | 0 | 1 | 0 | 0 | 0 | 19.32 | 25.3 |
| 1,3-dioxolane,2-(phenylmethyl)- | C1COC(O1)Cc1ccccc1 | $C_{10}H_{12}O_2$ | 164.2 | 12 | 6 | 0.4 | 2 | 2 | 0 | 45.89 | 18.46 |
| n- Allyloxymethyl acrylamide | C=CCOCNC(=O)C=C | $C_7H_{11}NO_2$ | 141.17 | 10 | 0 | 0.29 | 6 | 2 | 1 | 38.9 | 38.33 |
| Silane, [1-(5-hexenyl)-2methylenecyclopropy | C[Si](C1C2CC3CC1CC(C2)C3)(C)C | Low | 208.42 | 14 | 0 | 1 | 1 | 0 | 0 | 66.13 | 0 |
| 14-Heptadecenal | O=CCCCCCCCCCCCCCC=CCC | High | 252.44 | 18 | 0 | 0.82 | 14 | 1 | 0 | 83.56 | 17.07 |
| 4,6-Decadiyn-3-ol, 3-isopropyl-9-(methoxyeth | COCCOCOC(CC#CC#CC(C(C)C)(CC)O)C | High | 296.4 | 21 | 0 | 0.76 | 9 | 4 | 1 | 84.61 | 47.92 |
| Silicate anion tetramer | C[Si](O[Si]1(C)O[Si](C)(O[Si](C)(C)C)O[Si](C)(O[Si](C)(C)C)O[Si](O[Si](O[Si](O1)(C)O[Si](C)(C)C)(C)O[Si](C)(C)C)(C)O[Si](C)(C)C)(C)C | Low | 889.85 | 48 | 0 | 1 | 12 | 12 | 0 | 222.8 | 110.76 |

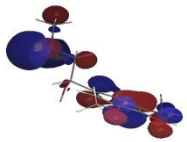
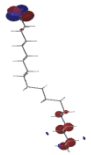
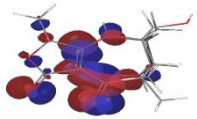
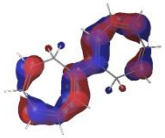
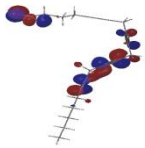
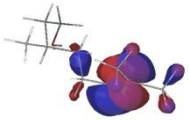
| Compound Name | Ali Solubility (mg/ml) | Ali Solubility (mol/l) | Ali Class | Silicos-IT LogSw | Silicos-IT Solubility (mg/ml) | Silicos-IT Solubility (mol/l) | Silicos-IT class | Ghose #violations | Veber #violations | Egan #violations | Muegge #violations |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Hepten-3-one,4-methyl- (CAS) 4-Methyl | 1.16 | 0.00918 | Soluble | -1.85 | 1.79 | 0.0142 | Soluble | 1 | 0 | 0 | 2 |
| 1,2,3-Propanetriol, diacetate (CAS) Diacetin | 31.4 | 0.178 | Very soluble | -0.31 | 85.7 | 0.486 | Soluble | 2 | 0 | 0 | 1 |
| Cytidine (CAS) Cyd | 198 | 0.816 | Very soluble | 1.01 | 2490 | 10.2 | Soluble | 1 | 0 | 0 | 1 |
| Zingiberene | 0.00248 | 1.21E-05 | Moderately soluble | -3.1 | 0.162 | 0.000791 | Soluble | 0 | 0 | 0 | 2 |
| Hexadecanoicacid (CAS) Palmitic acid | 4.31E-06 | 1.68E-08 | Poorly soluble | -5.31 | 0.00125 | 4.88E-06 | Moderately soluble | 0 | 1 | 0 | 1 |
| Benzoldicarbonsaeure | 0.0661 | 0.0002 | Soluble | -3.26 | 0.182 | 0.000551 | Soluble | 0 | 0 | 0 | 0 |
| Dodecanoic acid (CAS) Lauric acid | 0.00406 | 2.03E-05 | Moderately soluble | -3.69 | 0.0405 | 0.000202 | Soluble | 0 | 0 | 0 | 0 |
| Cyclopentane, heneicosyl- (CAS) Heneicosane | 5.02E-12 | 1.38E-14 | Insoluble | -9.47 | 1.24E-07 | 3.39E-10 | Poorly soluble | 2 | 1 | 1 | 3 |
| 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | 0.11 | 0.000776 | Soluble | -2.19 | 0.929 | 0.00653 | Soluble | 1 | 0 | 0 | 2 |
| 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | 11.9 | 0.0525 | Very soluble | -2.4 | 0.898 | 0.00395 | Soluble | 0 | 0 | 0 | 0 |
| Nonadecanoic Acid | 1.15E-07 | 3.85E-10 | Poorly soluble | -6.51 | 9.18E-05 | 3.08E-07 | Poorly soluble | 1 | 1 | 1 | 2 |
| Phthalic acid, butyl ester, ester with butyl glyc | 0.517 | 0.00154 | Soluble | -2.07 | 2.85 | 0.00847 | Soluble | 0 | 0 | 0 | 0 |
| Bi-1,3,5-cycloheptatrien-1-yl (CAS) | 0.0265 | 0.000146 | Soluble | -1.4 | 7.24 | 0.0397 | Soluble | 0 | 0 | 0 | 2 |
| 5,8,11,14-Icosatetraynoic Acid | 0.000445 | 1.50E-06 | Moderately soluble | -4.01 | 0.0289 | 9.75E-05 | Moderately soluble | 0 | 0 | 0 | 1 |
| dl-Citronellol | 0.0145 | 9.26E-05 | Moderately soluble | -2.21 | 0.964 | 0.00617 | Soluble | 1 | 0 | 0 | 2 |
| 1, E-11, Z-13-Octadecatriene | 2.88E-06 | 1.16E-08 | Poorly soluble | -5.35 | 0.00111 | 4.46E-06 | Moderately soluble | 1 | 1 | 1 | 2 |
| 9,12,15-Octadecatrienoic acid, methyl ester | 6.85E-05 | 2.34E-07 | Poorly soluble | -4.65 | 0.00649 | 2.22E-05 | Moderately soluble | 1 | 1 | 0 | 1 |
| 9-Octadecenoic acid (Z)- (CAS) Oleic acid | 1.54E-06 | 5.46E-09 | Poorly soluble | -5.39 | 0.00114 | 4.04E-06 | Moderately soluble | 1 | 1 | 1 | 1 |
| Trans-2-Phenyl-1,3-Dioxolane-4-M | 8.17E-08 | 1.85E-10 | Poorly soluble | -6.34 | 0.000202 | 4.59E-07 | Poorly soluble | 3 | 1 | 1 | 1 |
| 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | 1.60E-06 | 4.11E-09 | Poorly soluble | -7.4 | 1.56E-05 | 3.99E-08 | Poorly soluble | 1 | 1 | 1 | 2 |
| Docosane | 9.47E-10 | 3.05E-12 | Insoluble | -8.73 | 5.73E-07 | 1.85E-09 | Poorly soluble | 1 | 1 | 1 | 3 |
| Phthalic acid, di-(1-hexen-5-yl) ester | 0.000451 | 1.36E-06 | Moderately soluble | -5.13 | 0.00244 | 7.38E-06 | Moderately soluble | 0 | 1 | 0 | 1 |
| Propanoyl chloride | 5.89 | 0.0636 | Very soluble | -1.14 | 6.64 | 0.0718 | Soluble | 3 | 0 | 0 | 3 |
| Methane, Thiobis- | 6.28 | 0.101 | Very soluble | -0.6 | 15.7 | 0.253 | Soluble | 3 | 0 | 0 | 3 |
| 1,3-dioxolane,2-(phenylmethyl)- | 2.44 | 0.0149 | Very soluble | -2.87 | 0.22 | 0.00134 | Soluble | 0 | 0 | 0 | 1 |
| n- Allyloxymethyl acrylamide | 11.1 | 0.0788 | Very soluble | -1.3 | 7.12 | 0.0505 | Soluble | 2 | 0 | 0 | 1 |
| Silane, [1-(5-hexenyl)-2methylenecyclopropy | 0.00153 | 7.34E-06 | Moderately soluble | -2.77 | 0.356 | 0.00171 | Soluble | 0 | 0 | 0 | 2 |
| 14-Heptadecenal | 3.81E-05 | 1.51E-07 | Poorly soluble | -5.57 | 0.000675 | 2.67E-06 | Moderately soluble | 1 | 1 | 0 | 2 |
| 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth | 0.153 | 0.000517 | Soluble | -2.79 | 0.482 | 0.00163 | Soluble | 0 | 0 | 0 | 0 |
| Silicate anion tetramer | 3.52E-12 | 3.96E-15 | Insoluble | -6.55 | 0.00025 | 2.81E-07 | Poorly soluble | 4 | 1 | 1 | 3 |

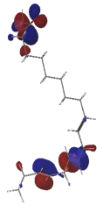
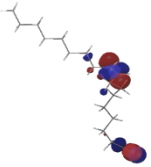
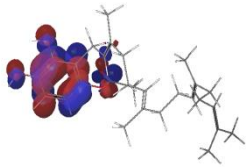
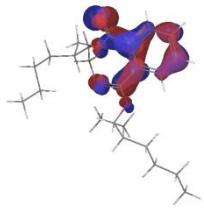
| Compound Name | GI Absorption | BBB permeant | Pgp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | log Kp (cm/s) | Lipinski #violations | Bioavailability Score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Hepten-3-one,4-methyl- (CAS) 4-Methyl | High | Yes | No | No | No | No | No | No | -5.61 | 0 | 0.55 |
| 1,2,3-Propanetriol, diacetate(CAS) Diacetin | High | No | No | No | No | No | No | No | -7.6 | 0 | 0.55 |
| Cytidine (CAS) Cyd | Low | No | No | No | No | No | No | No | -9.3 | 0 | 0.55 |
| Zingiberene | Low | No | No | No | Yes | Yes | No | No | -3.88 | 1 | 0.55 |
| Hexadecanoicacid (CAS) Palmitic acid | High | Yes | No | Yes | No | Yes | No | No | -2.77 | 1 | 0.85 |
| Benzoldicarbonsaeure | High | Yes | No | No | Yes | Yes | No | No | -6.39 | 0 | 0.85 |
| Dodecanoic acid (CAS) Lauric acid | High | Yes | No | No | No | No | No | No | -4.54 | 0 | 0.85 |
| Cyclopentane, heneicosyl- (CAS) Heneicosane | Low | No | Yes | No | No | No | No | No | 1.27 | 1 | 0.55 |
| 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | High | Yes | No | No | No | No | No | No | -5.02 | 0 | 0.55 |
| 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | High | No | No | No | No | No | No | No | -7.52 | 0 | 0.55 |
| Nonadecanoic Acid | High | No | No | Yes | No | No | No | No | -1.91 | 1 | 0.85 |
| Phthalic acid, butyl ester, ester with butyl glyc | High | No | Yes | No | No | No | No | No | -7.46 | 0 | 0.56 |
| Bi-1,3,5-cycloheptatrien-1-yl (CAS) | Low | Yes | No | Yes | No | Yes | No | No | -4.48 | 0 | 0.55 |
| 5,8,11,14-Icosatetraynoic Acid | High | Yes | No | Yes | Yes | Yes | No | No | -4.35 | 1 | 0.85 |
| dl-Citronellol | High | Yes | No | No | No | No | No | No | -4.48 | 0 | 0.55 |
| 1, E-11, Z-13-Octadecatriene | Low | No | No | Yes | No | Yes | No | No | -2.08 | 1 | 0.55 |
| 9,12,15-Octadecatrienoic acid, methyl ester | High | Yes | No | Yes | No | Yes | No | No | -3.62 | 1 | 0.55 |
| 9-Octadecenoic acid (Z)- (CAS) Oleic acid | High | No | No | Yes | No | Yes | No | No | -2.6 | 1 | 0.85 |
| Trans-2-Phenyl-1,3-Dioxolane-4-M | Low | No | No | No | No | Yes | No | Yes | -2.98 | 1 | 0.55 |
| 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | High | No | Yes | No | No | Yes | No | Yes | -3.39 | 1 | 0.55 |
| Docosane | Low | No | No | Yes | No | No | No | No | -0.01 | 1 | 0.55 |
| Phthalic acid, di-(1-hexen-5-yl) ester | High | Yes | Yes | No | Yes | Yes | No | Yes | -4.75 | 1 | 0.55 |
| Propanoyl chloride | High | Yes | No | No | No | No | No | No | -5.98 | 0 | 0.55 |
| Methane, Thiobis- | High | Yes | No | No | No | No | No | No | -6.05 | 0 | 0.55 |
| 1,3-dioxolane,2-(phenylmethyl)- | High | Yes | No | No | No | No | No | No | -6.01 | 0 | 0.55 |
| n- Allyloxymethyl acrylamide | High | Yes | No | No | No | No | No | No | -6.65 | 0 | 0.55 |
| Silane, [1-(5-hexenyl)-2methylenecyclopropy | Low | Yes | No | No | Yes | Yes | No | No | -3.75 | 1 | 0.55 |
| 14-Heptadecenal | High | No | No | Yes | No | No | No | No | -3.11 | 1 | 0.55 |
| 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth | High | Yes | Yes | No | No | No | No | No | -6.24 | 0 | 0.55 |
| Silicate anion tetramer | Low | No | Yes | No | No | No | No | No | -3.16 | 2 | 0.17 |

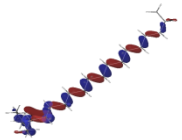
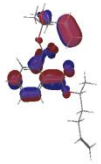
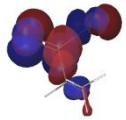
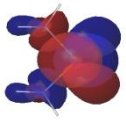
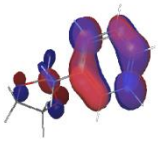
**TABLE 18: HOMO – LUMO Prediction of Ligands**

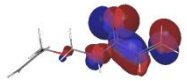
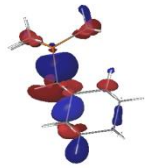
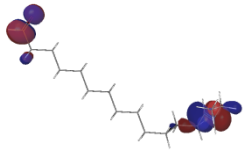
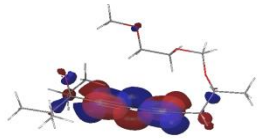
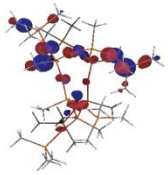
| S. No | CID | Compound Name | Solvation energy | Images | Homo | Lumo | HLG (eV) |
|---|---|---|---|---|---|---|---|
| 1. | 5362893 | 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl | 3.244 |  | -0.23792 | -0.04803 | -0.18989 |
| 2. | 66021 | 1,2,3-Propanetriol, diacetate (CAS) Diacetin | -2.657 |  | -0.26606 | 0.00149 | -0.26755 |
| 3. | 6175 | Cytidine (CAS) Cyd | 29.063 |  | -0.22567 | -0.0267 | -0.19897 |
| 4. | 92776 | Zingiberene | 2.464 |  | -0.19671 | -0.01326 | -0.18345 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5. | 985 | Hexadecanoic acid (CAS) Palmitic acid | 9.737 |  | 0.00863 | 0.12977 | -0.12114 |
| 6. | 5358705 | Benzoldicarbonsaeure | 7.981 |  | -0.03573 | 0.03593 | -0.07166 |
| 7. | 3893 | Dodecanoic acid (CAS) Lauric acid | 2.878 |  | 0.00608 | 0.1498 | -0.14372 |
| 8. | 23171 | Cyclopentane, heneicosyl- (CAS) Heneicosane | 24.964 |  | -0.27716 | 0.08402 | -0.36118 |
| 9. | 5364941 | 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | 1.506 |  | -0.24686 | -0.01963 | -0.22723 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 10. | 31513 | 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | 27.392 |  | 0.29267 | -0.04931 | 0.34198 |
| 11. | 12591 | Nonadecnoic Acid | 11.041 |  | 0.28345 | -0.06574 | 0.21771 |
| 12. | 3074876 | Phthalic acid, butyl ester, ester with butyl glyc | 23.78 |  | -0.22533 | -0.05209 | -0.17324 |
| 13. | 563003 | Bi-1,3,5-cycloheptatrien-1-yl (CAS) | 23.402 |  | -0.18258 | -0.07431 | -0.10827 |
| 14. | 1780 | 5,8,11,14-Icosatetraynoic Acid | 1.891 |  | 0.00299 | 0.09957 | -0.09658 |
| 15. | 8842 | dl-Citronellol | -0.262 |  | -0.2231 | 0.03433 | -0.25743 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 16. | 5365585 | 1, E-11, Z-13-Octadecatriene | -1.084 |  | -0.20809 | -0.01216 | -0.19593 |
| 17. | 5367462 | 9,12,15-Octadecatrienoic acid, methyl ester | -3.94 |  | -0.23015 | 0.00902 | -0.23917 |
| 18. | 445639 | 9-Octadecenoic acid (Z)- (CAS) Oleic acid | 2.677 |  | -0.00523 | 0.09333 | -0.09856 |
| 19. | 5387599 | Trans-2-Phenyl-1,3-Dioxolane-4-M | 13.755 |  | -0.20891 | -0.00073 | -0.20818 |
| 20. | 7057919 | 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | 23.243 |  | -0.25579 | -0.05531 | -0.20048 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21. | 12405 | Docosane | 11.617 |  | -0.27736 | 0.08274 | -0.3601 |
| 22. | 590929 | Phthalic acid, di-(1-hexen-5-yl) ester | 11.276 |  | -0.24487 | -0.05693 | -0.18794 |
| 23. | 62324 | Propanoyl chloride | 1.256 |  | -0.29267 | -0.04931 | -0.24336 |
| 24. | 1068 | Methane, Thiobis- | 0.377 |  | -0.21583 | 0.04826 | -0.26409 |
| 25. | 7562 | 1,3-dioxolane, 2-(phenylmethyl)- | -3.299 |  | -0.23335 | 0.00452 | -0.23787 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 26. | 19357475 | n- Allyloxymethyl acrylamide | 6.649 |  | -0.24366 | -0.03824 | -0.20542 |
| 27. | 13131343 | Silane, [1-(5-hexenyl)-2methylenecyclopropy | 22.771 |  | -0.24638 | 0.0629 | -0.30928 |
| 28. | 5367667 | 14-Heptadecenal | 1.801 |  | -0.23477 | -0.02521 | -0.20956 |
| 29. | 547946 | 4,6-Decadiyn-3-ol, 3-isopropyl-9-(methoxyeth | 7.064 |  | -0.23168 | -0.01353 | -0.21815 |
| 30. | 129801358 | Silicate anion tetramer | -154.293 |  | -0.27021 | 0.02081 | -0.29102 |

# 5. Discussion

## 5.1 Phytochemical Screening of Bioactive Compounds from the Leaves and Barks of *Pterocarpus indicus*

The presence of various phytochemical compounds viz., tannin, phlobatannin, saponin, flavonoids, steroids, terpenoids, anthocyanin, anthraquinone, coumarin, phenol, cardiac glycoside, xanthoprotein, alkaloids, emodin and carbohydrates were analyzed in ethanolic extracts of leaves and barks of *Pterocarpus indicus* (Table 1). The previous studies suggested that the barks of *Pterocarpus indicus* revealed the presence of various bioactive compounds such as tannins, phenols, terpenoids, sterols, protein, anthocyanin, carbohydrate, flavonoids and steroids (Senthil Kumar *et al.*, 2020).

Patil and Gaikwad (2011) reported that the methanolic extract of stem bark of *Pterocarpus marsupium* showed the presence of glycosides, flavonoids, phenols, alkaloids, flavanols and terpenoids. The hexane, ethyl acetate and methanol extracts of bark and leaf of *Pterocarpus marsupium* revealed the presence of sugar, protein, flavonoids, saponin, cardiac glycoside, lipids and tannins (Ramya, 2008).

Sarker (2012) suggested that the phytochemical analysis of *Pterocarpus santalinus* showed carbohydrate, anthocyanins, tannins, phenols, terpenoids, flavonoids, glycosides and glycerides. Challa *et al.*, (2019) demonstrated that the aqueous and ethanolic extracts of leaves of *Pterocarpus santalinus* revealed the presence of proteins, carbohydrates and tannins. Usunomena and Chinwe (2016) reported that the leaves of *Pterocarpus mildbraedii* showed the presence of saponins, alkaloids, tannins and flavonoids.

## 5.2 Quantitative Estimation of Important Secondary Metabolites from the Leaves and Barks of *Pterocarpus indicus*

The ethanolic solvent extracts contained the maximum quantity of phytochemicals (as evident from qualitative screening) were selected for the quantification of secondary metabolites. The phytochemicals with the highest quantity of active compounds were quantified by leaves showed flavonoids (0.036mg/g), tannin (0.083mg/g), saponin (0.051mg/g), alkaloids (0.057mg/g), phenols (0.024mg/g), terpenoids (0.028mg/g) respectively. The barks of *Pterocarpus indicus* showed tannin (0.053) followed by flavonoids (0.018mg/g), saponin (0.020mg/g), alkaloids (0.025mg/g), phenols (0.010mg/g), terpenoids (0.012mg/g) respectively (Table 2). The previous literature demonstrated that the *Pterocarpus marsupium* contains the various phytocompounds such as pterosupin, marsupsin, epicatechin and pterostilbene (Gairola *et al.*, 2010).

Pant *et al.*, (2017) reported that the total phenol content of acetone extract was 38.01 mg GAE/g and ethanol extract was 59.42 mg GAE/g and for the total flavonoids content acetone extract was 82. 56 mg GAE/g and ethanol extract were 38.56 mg GAE/g of *Pterocarpus marsupium.* Kesari *et al.*, (2004) reported that the *Pterocarpus* species are found to be rich in terpenoids, lupeol, beta- sitosterol, epicatechin and isoflavonoids. Alkalis, ester, santal, homopterocarpin, pterocarpin, tannin and ketotannic acid were quantified from the wood of *Pterocarpus santlinus* (Krishna Veni and Srinivasa Rao, 2000).

## 5.3 Synthesis and Characterization of Silver Nanoparticles using Leaves and Stem Bark of *Pterocarpus indicus*

The experiment was carried out by adding ethanol in leaves and barks extract of *Pterocarpus indicus* each separately add in to the glass vial which contains $AgNO_3$, the

change in color from colorless to reddish brown for barks extract and for leaves the gradual change in the colour of samples from light green to dark brown colour was observed which denotes the presence of silver nanoparticles. The presence of silver nanoparticles was viewed by using the UV-Vis spectral technique at room temperature. The UV visible absorption spectrum was noted at the range of 435 nm for the leaves-silver nanoparticles (Figure 2a) and at the range of 439 nm for the stem bark-silver nanoparticles (Figure 2b). The previous studies reported that the silver nanoparticles are known to shows the UV-Visible absorption in the range 400-500 nm and also showed the sharp absorption bands at 431 nm for *Pterocarpus marsupium* silver nanoparticles (Zhang *et al.*, 2016). Jadhav *et al.*, (2016) demonstrated that the development of silver nanoparticles using *Pterocarpus indicus* was observed by the colour change from light yellow to dark brown and also the absorption spectra of silver nanoparticles have absorption peaks at 420 nm. The UV Visible spectra of *Pterocarpus marsupium* was observed for the absorption peak at 279 nm were taken in the range of 200-800 nm (Bagyalakshmi and Haritha, 2017). The aqueous leaves extract of *Pterocarpus santalinus* showed the formation of silver nanoparticles in the extracts is observed by its colour change brown colour and the peak was observed at 420 nm in the UV spectrophotometer.

The FT-IR spectrum of synthesized silver nanoparticles gives information about the functional group involved in the silver ions reduction. The following peaks are observed in the leaves-AgNPs, the medium band primary amine at 3438.11 $cm^{-1}$ corresponds to N-H stretching vibrations. The strong band Isothiocyanate at 2085.17 $cm^{-1}$ corresponds to N=C=S stretching vibrations. The medium band Alkene at 1637.86 $cm^{-1}$ corresponds to C=C stretching vibrations, Amine at 1109.08 $cm^{-1}$ corresponds to C-N stretching vibrations. The Strong band Halo compound at 670.15 $cm^{-1}$ corresponds to C-Br stretching vibrations (Figure 3a). For the stem bark-

AgNPs, the strong band primary amine at 3441.16 $cm^{-1}$ corresponds to 3441.16 $cm^{-1}$ corresponds to broad O-H stretching alcohol. The Strong band Isothiocyanate at 2077.79 $cm^{-1}$ corresponds to N=C=S stretching vibrations. The Medium band Alkene at 1634.81 $cm^{-1}$ corresponds to C=C stretching conjugated alkene and amine at 1405.54 $cm^{-1}$ corresponds to O-H bending alcohol.

The Strong band Alcohol at 1114.45 $cm^{-1}$ corresponds to C=O secondary alcohol, Anhydride at 1030.7 $cm^{-1}$ corresponds to CO-O-CO anhydride and Halo compound at 662.74 $cm^{-1}$ corresponds to C-Br stretching vibrations (Figure 3b).

Analysis of these spectra strongly suggested the presence of flavonoids and phenols, which were mainly responsible for the formation of silver nanoparticles by reducing silver nitrate. The previous studies suggested that the active compounds such as saponins, phenols, terpenoids, flavonoids, glycosides, steroids and tannins were observed in the leaves of *Pterocarpus santalinus* are responsible for the reduction and capping during the synthesis of silver nanoparticles (Kumar *et al.*, 2012). Dhivya and Rajasimman (2015) and (Sikarwar *et al.*, 2008) reported that the hydroxyl, carboxyl and phenolic groups present in the medicinal plants may act as reducing, stabilizing and capping agents.

Zhang *et al.*, (2003) reported that the higher peaks at 3438 and 3441 cm-1 might be attributed to hydroxyl (OH) of alcohol/phenols or amine (-NH) groups of silver nanoparticles and these bonds could be due to the hydroxyl group stretching in enzymes, proteins or polysaccharides found in the *Pterocarpus indicus*. The lower bands at 670 and 662 cm-1 are due to the alkyl halides found in anthocyanin and flavonoids present in the leaves and barks of *Pterocarpus indicus* (Monga *et al.*, 2013). Diantoro *et al.*, (2018) demonstrated that the leaves extract of *Pterocarpus indicus* showed a peak transmittance

of 3358, 2946, 2834, 1449. 1111, 636,535 cm-1 wave number and these peaks are responsible of quercetin flavonoids compounds. The *Pterocarpus indicus* and *Jatropha multifida* leaves and stem extract of plants are good sources as natural polymers.

The FTIR analysis showed that the peaks correspond to aromatic CH functional groups, C=C aromatic, aliphatic CH and –OH, aromatic CO due to the presence of flavonoids compounds rutin present in the polymers of leaves and stem of plants extracts.

Bagyalakshmi and Haritha (2017) reported that the bark and wood of *Pterocarpus marsupium* showed the FTIR spectrum of absorption peaks at 3693 cm-1 indicates OH stretching due to the presence of phenol and alcohol. The peaks at 1619 and 1530 cm-1 indicates C=C stretching of alpha and beta unsaturated ketone and the peaks at 1384 and 821 cm-1 showed the presence of alkenes and aromatic rings.

In X-ray crystallography the crystalline nature of silver nanoparticles was confirmed using the leaves and stem bark of *Pterocarpus indicus* (Table 3&4). The synthesized silver nanoparticles pattern performed by XRD. The diffraction peaks were obtained by leaves-AgNPs is observed at 38.4024, 46.3896, 65.1721 and 78.568 in the 2Ɵ range (Figure 4a). The obtained XRD pattern for silver nanoparticles synthesized using *Pterocarpus indicus* bark extract showed the characteristic peaks 38.7965, 44.7206, 65.0686 and 78.2134 in the 2Ɵ range respectively (Figure 4b).

The pattern (111), (200), (220) and (311) observed was face cantered cubic structure for silver according to (JCPDS, File No. 04-0783). The peaks obtained from the graph was unassigned may be due to extract contains some phytochemicals which may be capping the nanoparticles surface. The previous literature suggested that the XRD pattern demonstrated that the silver nanoparticles formed by the reduction of silver ions using the *Pterocarpus santalinus* leaves extract due to the crystalline in nature (Raman

*et al.*, 2012). The XRD patterns of dried synthesized silver nanoparticles using fluoresced peaks at 2θ values of 21.64º, 29.48º, 38.84º, 43.28º and 53.48º assigned to the (200), (101), (144), (202) and (311) planes of a faced centre cubic lattice of silver (Abid *et al.*, 2002). XRD results reported that the crystallization of the bio-organic phase occurs on the plane of the synthesized silver nanoparticles (Eutis *et al.*, 2005).

Diantoro *et al.*, (2018) suggested that the XRD data showed an increase in the crystalline of the flavonoids *Pterocarpus indicus* silver nanoparticles film indicated by the peaks at 38.51º of (111) Bragg's plane. Gazal *et al.*, (2004) showed that the flavonoids Pterocarpus indicus silver nanoparticles produces a peak at 2θ of 38.12º and also observed that there is a small shift of the XRD patterns due to the presence of flavonoids contents in the synthesized silver nanoparticles.

SEM image reveals most of the synthesized silver nanoparticle was spherical in shape and well dispersed (Figure 5). From the SEM image it was concluded that synthesized silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* were almost uniform in shape and size. SEM images of the synthesized silver nanoparticles lie between 37.9-124.5 nm region in case of leaves-AgNPs (Figure 5a) and 98.70-126 nm in case of stem bark-AgNPs sample (Figure 5b), the average size of the nanoparticles is ~ 200 nm, whereas the shapes were spherical and cubic.

From the previous studies reported that the SEM analysis showed that the synthesized silver nanoparticles using *Pterocarpus marsupium* have spherical in shape and assembling of silver nanoparticles on the surface and also reported that the synthesized silver nanoparticles are predominantly spherical in morphology with sizes range from 200 to 300 nm. SEM images was used to find the morphology of the synthesized silver nanoparticles are cluster in shape with an average size range from 20-

100 nm and also confirmed that the formation of silver nanoparticles capped with its biomolecules due to surface plasmon resonance (Dawidowicz and Olszowy, 2012). The SEM results showed that the synthesized silver nanoparticles using *Pterocarpus indicus* high in flavonoids contents showed that the silver nanoparticles exhibit their size range from 30 nm to 50 nm (Diantoro *et al.*, 2018).

Particle size distribution analyzer is performed to analysis the particle size distribution, average size of the nanoparticles and polydispersity index (PDI) of the synthesized silver nanoparticles. Dynamic light scattering results also confirmed an average size of the biosynthesized silver nanoparticles was in nanometre range. The intensity weighed particle size distribution histograms are obtained from the leaves-AgNPs exhibited polydisperse mixture with the size ranging of 1842 nm with poly dispersity index of 0.247 (Figure 6a) and for the stem bark-AgNPs exhibited polydisperse mixture with the size ranging of 385 nm with poly dispersity index of 0.063 (Figure 7a).

The zeta potential is used to depict the surface charge and stability of synthesized silver nanoparticles using *Pterocarpus indicus*. For leaves-AgNPs zeta potential measured was found to be −13.1 mV with peak area of 100% intensity (Figure 6b). The biosynthesized stem bark-AgNPs had a negative charge with a zeta potential value −16.3 mV (Figure 7b). This zeta potential value falls within the range of −20 to −30 mV is considered as moderately stable, which clearly indicated that the synthesized S-AgNPs was moderately stable in nature (Table 5 & 6).

From the average particle size and PDI value it is found that produced nanoparticles are monodispersed in nature. The previous studies by Vitthal *et al.*, (2013) reported that the zeta sizer is used to analysis the particle size was found to be 148.5 nm with polydispersity index 0.336 and intercept 0.963 and for zeta potential was found to

be -28 mV with peak area of 100% intensity. These values suggested that the stabilization of synthesized silver nanoparticles using *Pterocarpus marsupium*. Galati *et al.*, (2000) reported that the high negative potential value that led to increase attraction force negative-negative repulsion between silver nanoparticles which in turn lead to long stability, nanoparticle aggregation and gold colloidal in nature.

Manne *et al.*, (2020) suggested that the chitosan nanoparticles synthesized using *Pterocarpus marsupium* exhibited zeta potentials of 40.4, 57.3, 44.6 mV and for chitosan nanoparticles without the plant extracts had a positive charge of 36.2 mV occurs due to the rise in groups of positive charges on the surface of plant extracts synthesized chitosan nanoparticles are relatively stable.

Agarwal *et al.*, (2018) suggested that the silver nanoparticles with a zeta potential > ±30 mV are stable in nature as the surface charge inhibits particles aggregation. Umoren *et al.*, (2014) demonstrated that the zeta potential of synthesized silver nanoparticles was found to be -65.07 mV and as revealed by the negative value of zeta potential due to the presence of natural products and also showed the stability of the formation and average size of the silver nanoparticles is 150 nm.

## 5.4 Antidiabetic Activity of Synthesized Silver Nanoparticles by using Leaves and Stem Bark of *Pterocarpus indicus* and compared with Standard Drug Acarbose

The synthesized silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* were investigated by alpha amylase inhibitory activities under *in vitro* conditions (Chart: 1). The synthesized silver nanoparticles of the various concentrations (20-100 µg/ml) exhibited potent alpha amylase inhibitory activity in a dose dependent manner.

The silver nanoparticles using *Pterocarpus indicus* extracts showed inhibitory activity from 56.95% to 67.75% for leaves and for the stem 65.95% to 80.36% at a concentration 100 µg/ml. Acarbose is a standard drug and showed the inhibitory activity from 70.27% to 82.88% at a concentration 100 µg/ml has been depicted (Table 7).

The synthesized silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* showed the antidiabetic activity using alpha glucosidase inhibitory activity (Chart: 2) from 58.74% to 72.85% for leaves and from 48.83% to 64.64% for stem bark at a concentration 100 µg/ml. Acarbose is a standard drug at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 70.270% to 82.882% at the same concentrations 100 µg/ml (Table 8). Thus, the inhibition of the activity of alpha glucosidase by synthesized silver nanoparticles would delay the degradation of carbohydrate, which in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation.

The previous literature suggested that the synthesized silver nanoparticles using *Pterocarpus marsupium* Roxb. is used to analyse the hypoglycemic effects by *in vitro* alpha amylase inhibition model. The inhibitory effects of *Pterocarpus marsupium* latex were showed IC50 of 2.97 for alpha amylase and for alpha glucosidase 0.54 µg/ml by *in vitro* study (Poongunran *et al.,* 2015). The aqueous extract of latex of *Pterocarpus marsupium* had showed the inhibitory activity on alpha glucosidase activity (Maruthupandian and Mohan, 2011).

Gayathri and Kannabiran (2010) reported that the administration of bark of *Pterocarpus marsupium* to diabetic rats solved the level of serum and glycolytic enzymes by inhibiting the formation of kidney and liver lipid peroxides. Vats *et al.,* (2002) reported that the aqueous extract of bark of *Pterocarpus marsupium* reduced the blood

sugar levels from 202.91% to 85.22% mg 21 days after oral administration of the extracts in both normal and alloxan induced diabetic rats. Zaid *et al.,* (2002) demonstrated that the bark of *Pterocarpus marsupium* is an antidiabetic drug due to the presence of compounds epicatechin belonging to the class of flavonoids family.

Maheswari *et al.*, (1980) reported that the patients of diabetic mellitus used as a traditional therapy of overnight water stored in water tumblers made out of the heartwood of *Pterocarpus marsupium.*

Bagyalakshmi and Haritha (2017) suggested that the synthesized silver nanoparticles using *Pterocarpus marsupium* showed good alpha amylase inhibition with IC50 value of 700 µg/ml as compared to positive control acarbose IC50 value 180 µg/ml. Sichaem *et al.*, (2018) investigated that the barks of *Pterocarpus indicus* led to isolated triterpenoids compounds and were evaluated for alpha glucosidase activity and showed the potent inhibitory activity with an IC 50 value of 37.2 and 39.8 µM, respectively.

## 5.5 Cytotoxicity Activity of Synthesized Silver Nanoparticles using Leaves and Stem Bark of *Pterocarpus indicus* on Breast Cancer Cell Line MCF-7

The *in vitro* cytotoxicity study of the biosynthesized silver nanoparticles using leaves and barks of *Pterocarpus indicus* separately were investigated by an MTT assay against MCF-7 breast cancer cell line with different concentration 10-500 µg/ml to determine the IC50 (50% growth inhibition). The inhibitory concentration (IC50) value of biosynthesized silver nanoparticles using leaves is 54.60 µg/ml and for the stem barks-AgNPs is 126.4 µg/ml respectively (Table 9).

The stem barks- AgNPs was found more cytotoxic and also showed maximal antiproliferative effect as compared to the leaves-AgNPs on MCF-7 breast cancer cell lines. The previous studies also reported that the biosynthesized silver nanoparticles

showed high inhibitory effect against cancer cell lines with an increase in concentration of the tested samples (Selvi *et al.*, 2016). From the cell viability assay, the synthesized silver nanoparticles stem barks have more inhibitory effect to kill MCF-7 cancer cell and there is no drastic change when the incubation time is increased as compared to the leaves-AgNPs treated cells causes variation in cell viability when the time is increased at a concentration 400 and 500 µg/ml. The morphological changes of the breast cancer cell lines with various concentrations of leaves-AgNPs and stem bark-AgNPs of *Pterocarpus indicus* at incubation for 24 hours as compared with the untreated cells. After the incubation period, in the treated cancer cells the morphology of the synthesized silver nanoparticles using leaves and stem barks extracts significantly changed and apparent less uniform with the loss of membrane integrity at lower concentrations.

Whereas at higher concentrations 500 µg/ml, the synthesized silver nanoparticles treated cells showed significant changes such as karyopyknosis, loss of intact membrane, cell detachment from the plate and changes of morphological features as compared to the control cells without any additive (Figure; 9 & 10). The leaves-AgNPs and stem bark-AgNPs treated cells most identifiable morphological features of apoptosis were observed by inverted light microscopy. However untreated cells appeared normal and were confluent and for treated cells appeared cells undergoing apoptosis, detaching from the plate, cell shrinkage, condensation, loss of contact with neighbouring cells, and aggregation of the nuclear chromatin.

The previous studies suggested that the *Pterocarpus marsupium* contains phytoconstituents like pterostilbene, pterosupin, marsupin, epicatechin and liquiritigenin can be used for the treatment of breast and prostate cells (Katiyar *et al.*, 2016). Remsberg *et al.*, (2008) reported that the bioactive compounds pterostilbene from *Pterocarpus marsupium* was studied in HCT-116, Hep-G2, PC-23, A-375 and MDAMB-231 cell lines

by measuring cell viability. These compounds pterostilbene showed high activity against Hep-G2 (liver) IC50 value is 16.0 µM and for HCT-16 (colon) IC50 value is 45.3 µM cancers. Pan *et al.*, (2007) showed that the active constituents pterostilbene from *Pterocarpus marsupium* tested cell viability against human gastric carcinoma AGS cells and found that these compounds were able to kill cell proliferation and induce apoptosis.

Akhouri *et al.*, (2020) concluded that the *Pterocarpus santalinus* has therapeutic role against 7,12-dimethylbenz(a)-anthracene induced breast cancer in rats and also has a greater inhibitory effect to develop as a chemotherapeutic agent in the treatment of breast cancer. The phytochemicals compounds triterpenoids, sesquiterpenes, polyphenol, flavonoids, alkaloids from the *Pterocarpus santalinus* exhibited cytotoxic activity against various cancer cell lines (Cho *et al.*, 2001).

Donga *et al.*, (2017) suggested that the methanolic extract of leaf, stem and bark of *Pterocarpus santalinus* by cold percolation method showed cytotoxicity effect against breast cancer cell lines. The methanolic extract of heartwood extract of *Pterocarpus santalinus* contains active compounds such as pterolinus B, pterolinus D and s-30-hydroxyl-4,40-dimethoxydalbergione showed cytotoxic effect against breast cancer cell lines MCF-7 and MDA-MB-231 (Wu *et al.*, 2011).

## 5.6 Antioxidant Activity of Synthesized Silver Nanoparticles using Leaves and Stem Bark of *Pterocarpus indicus*

The DPPH results showed that the silver nanoparticles synthesized using a *Pterocarpus indicus* bark and leaf sample of exhibit antioxidant activities at high concentration when compared with standard ascorbic acid. The synthesized silver nanoparticles showed 70.149% (leaves-AgNPs) and 75.929 (stem bark-AgNPs) activity

at concentration 100 µg/ml while ascorbic acid gave 94.69 % at the same concentration (Table 10, Chart: 3).

Previous study suggested that the antioxidant activity of *Pterocarpus mildbraedii* leaves showed potent free radical scavenging activity ranged from 60.93% to 80.99% against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (Usunomena and Chinwe, 2016).

Tippani *et al.,* (2010) reported that the bark extract of *Pterocarpus marsupium* showed in vitro antioxidant activity was evaluated using the DPPH assay with the IC50 value 53µg/ml as compared with standard drug ascorbic acid IC50 is 34 µg/ml. The stem wood extract of *Pterocarpus marsupium* ethanol, isopropyl alcohol and acetone extracts each showed the antioxidant activities in DPPH radical scavenging method (Pant *et al.*, 2017). Abirami *et al.,* (2012) showed that the antioxidant activity of *Pterocarpus marsupium* bark extracts in DPPH, ABTS, superoxide, nitric oxide scavenging and hydroxyl radical.

The reducing power of ethanolic extracts of synthesized silver nanoparticles using leaves and bark of *Pterocarpus indicus* was performed and compared with standard ascorbic acid. Ethanolic extract of synthesized stem bark-AgNPs of *Pterocarpus indicus* (78.37%) exhibits good reducing power activity among leaves-AgNPs extracts (79.27%) as we compared to the standard drug ascorbic acid (84.68%) at a concentration 100 µg/ml (Table 11, Chart: 4).

The reducing power ability of a phytocompounds present in the leaves of *Pterocarpus mildbraedii* showed the significant activity depends on the presence of reductones which exert antioxidant activity by breaking the free radical by donating a hydrogen atom (Usunomena *et al.,* 2016). Mohammadi *et al.,* (2009) reported that the aqueous extract of stem bark of *Pterocarpus marsupium* showed high antioxidant activity

and protects the mitochondria against the oxidative damage. Kumaravel *et al.*, (2013) suggested that the ethyl acetate leaves extract of *Pterocarpus marsupium* showed the antioxidant activity was studied by using DPPH, ABTS assay, hydroxyl radical scavenging activity, reducing power assay and hydrogen peroxide radical activity under *in vitro* condition.

Joshi *et al.*, (2012) reported that the polyphenolic compounds like flavonoids, sesquiterpenes and diphenyl propane from heartwood juice of *Pterocarpus marsupium* showed antioxidant activities. Further many previous studies have showed that the different extract of *Pterocarpus marsupium* showed antioxidant, antidiabetic, anti-inflammatory, antimicrobial and analgesic activities (Roopashree *et al.*, 2008 and Mishra *et al.*, 2013). Gairola *et al.*, (2010) and Subba *et al.*, (2016) reported that the antioxidant activity of ethanol extract of wood and bark of *Pterocarpus marsupium* due to the presence of phenolic compounds.

## 5.7 Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds from the Leaves and Stem Bark of *Pterocarpus indicus*

GCMS is an important tool for the identification of bioactive compounds present in the leaves and bark of *Pterocarpus indicus* and also used to analysis their retention time, molecular formulae, molecular weight and structure of the active constituents present in the extracts (Table 12 & 13). So, in the present study, 10 bioactive compounds were identified from the ethanolic extracts of leaves and 20 bio active compounds from the stem barks of *Pterocarpus indicus* (Figure 11 & 12).

The previous study suggested that the aqueous extract of bark of *Pterocarpus marsupium* contains 27 phytocompounds such as Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]- trimethylsilyl ester, a-d-Mannofuranoside, methyl, Furan-2-

one,3,4-dihydroxy-5-[1-hydroxy-2-fluoroethyl], Phthalic acid, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methyl ethyl) with different therapeutic activities (Hugar *et al.*, 2017). Vats *et al.*, (2002) suggested that the *Pterocarpus indicus* contains marsupsin, epicatechin, kinotannic acid, pterostilbene have identified by GCMS analysis and also used for the treatment of cataract.

Hartati *et al.*, (2016) reported that the leaves *of Pterocarpus indicus* showed the presence of bio active compounds such as flavanol - glycoside [(2R)-7-hydroxy-3-(3, 4, 5- trihydroxy-6-(hydroxymethyl) tetrahydro-2Hpyran-2- yloxy)-2-(3,4,5-trihydroxy phenyl) chroman-4-one] or ptevon-3-D- glycoside were identified by GCMS analysis. The heartwood of Pterocarpus marsupium contains flavonoids C- glucosides namely 6 – hydroxyl – 2, 4 - hydroxybenzyl – benzofuran – 7C – β – D - glucopyranoside, 3 α – methoxy – 4 - hydroxybenzylidene – 6 – hydroxybenzo – 2(3H) – furanone – 7C – β- D - glucopyranoside, 2 glucopyranoside, 8 C – β - D - glucopyranosyl - 7, 3, 4- trihydroxy flavone and 1, 2 – bis (2, 4 - dihydroxy, 3 – C glucopyranosyl) – ethanedione, C-β-D-glucopyranosyl-2,6-dihydroxyl benzene and sesquiterpene were reported to have antioxidant actives.

The previous studies suggested that by using bark of *Pterocarpus indicus* revealed the twelve compounds including two major triterpenoids, one quinine derivative, three phenolic compounds, six flavonoids. In this regard, compound 1 lupeol and 2 canophyllol exhibited potent inhibitory activity against alpha glucosidase when compared to the acarbose (Sichaem *et al.*, 2018).

Yadav *et al.*, (2019) reported that the methanolic extract of heartwood of *Pterocarpus marsupium* indicated that the hydrogen bonding between liquiritigenin and catalytic triad (Asp 197. Glu 233, ASP 300) of alpha amylase and HIS 407 of alpha

glucosidase with -5.60 and -7.10 binding energies by molecular docking study. Challa *et al.*, (2019) suggested that the twenty compounds identified from methanolic extract of *Pterocarpus santalinus* by GCMS were used for the docking study showed that the compounds cyclo hexane, 1-ethyl-1-methyl, 2,4-bis (1-methyl, ethanyl) shown best docking energy to the alpha glucosidase.

### 5.8 Molecular Docking Analysis of Alpha Amylase and Alpha Glucosidase

Docking studies by Maestro (Schrodinger) showed that the compounds cytidine (CAS) cyd had the highest docking score (-7.998) followed by 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon (-7.222), benzoldicar bonsaeure (-6.093), 1,2-benzenedicarboxylic acid, bis (2-ethylhexy) (-5.689) against alpha amylase enzymes acarbose which is -14.339 KJ/MOL which may be a potent anti-diabetic compounds because the high docking score, the compound cytidine (CAS) cyd will be potent antidiabetic drugs against alpha amylase activity (Table 14). Acarbose docking with alpha amylase indicted that the model structure of ligand-protein interaction between acarbose and alpha amylase had formed interaction of ligand molecules (Figure 13).

Docking studies by Maestro (Schrodinger) showed that the compounds 2-(4-hydroxyl-2-butenyl)-2-nitrocyclo heptanon (-7.496) followed by trans-2-phenyl-1,3-dioxolane-4 (-6.472), cytidine (CAS) cyd (-6.233), phthalic acid, butyl ester, ester with butyl glyc (-5.036) against alpha glucosidase enzymes acarbose which is -14.452 KJ/MOL which may be a potent anti-diabetic compounds because the high docking score, the compound 2-(4-hydroxyl-2-butenyl)-2-nitrocyclo heptanon will be potent antidiabetic drugs against alpha glucosidase activity (Table 15). Acarbose docking with alpha glucosidase indicted that the model structure of ligand-protein interaction between acarbose and alpha glucosidase had formed interaction of ligand molecules (Figure 15).

The previous study suggested that the docking scores for acarbose yields better inhibition with binding free energy of about -8.2 to -11.9 kcal/mol and for capsicum provided the binding score -5.8 to -6.1 kcal. mol. The amino acids such as Tyr82, Asp340, Tyr155 and Asp206 in the pockets of amylase and glucosidase have been previously identified as the common amino acids stabilizing the interaction of the two enzymes with ligands of antidiabetic drugs (Thongnum and Chanthai 2018; Sanni *et al.*, 2019).

### 5.9 Molecular Docking Analysis of Human Estrogen Receptor Alpha (ER-α)

Grid based docking study was used to analysis the binding modes of molecules with the amino acids present in the active side of the protein in order to study the interaction of the compounds with the estrogen receptor alpha (ER-α) (Figure 17). We also performed glide docking analysis by Schrodinger, where compared to all the compounds phthalic acid, butyl ester, ester with butyl glyc (-8.827) followed by cytidine (CAS) cyd (-8.781), Bi-1,3-5-cyclo heptatrien-1-yl (CAS) (-8.353) showed the best docking score as shown in (Table 16) due to the strong binding bond between 3 ERT and compounds in favourable conformation.

Tamoxifen (-12.982) is an antagonist of ER-alpha used to control the breast cancer and also it binds the amino acids and blocks the function of estrogen receptor and inhibits the function of human estrogen receptor.

The previous study suggested that the compounds pterostilbene purified from *Pterocarpus marsupium* was conducted to test the anti-cancer activity on breast cancer cell line MCF-7 and prostate PC3 cancer cell lines. The compounds have multiple target sites to induce apoptosis and also as a potential agent for the cure of breast and prostate cancer (Ajanta Chakraborty *et al.*, 2010).

# 6. Summary and Conclusion

Nanomedicine is research in multidisciplinary field they mainly use nanomaterials for designing nanomedicine. Nanoparticles because of their size dependent features there are very essential in several natural associated activities and human welfare. Current trend is based on herbal nanomedicine which is becoming a frontier in the nano formulation research field. Recent scientific research proves that herbal nanomedicine is very useful for the treatment of various diseases. The nanoparticles which are obtained from biological based method show high stability, high yield, and solubility. Green synthesis of nanoparticles is an easy, efficient, non- toxic and eco-friendly method. It consumes low energy and produces safer products and by products. There are several noble metal nanoparticles but silver nanoparticles have been attracted because of their unique properties like chemical stability, electrical conductivity and catalytic activities when compared with bulk materials.

Diabetes mellitus and breast cancer are most prevalent chronic diseases among women. Approximately 16% of breast cancer patients suffered from diabetes. Diabetes mellitus has been identified as an independent risk factor for breast cancer. A well-designed meta-analysis suggested that the women with diabetes had a 23% greater risk of subsequent breast cancer. Pre-existing diabetes is associated with a 37% greater risk of breast cancer mortality in female breast cancer patients, respectively. The current cytotoxic agents worn for the breast cancer treatment are highly expensive and not efficient because of their side effects due to the toxic nature of the non-cancerous tissues. Today the most efficient nanoparticles used to treat cancer cells are the silver nano particles, it is a biologically synthesised active nanoparticle against human breast cancer.

The present research work was carried out to prove the medicinal properties and significance of *Pterocarpus indicus* Willd. which is being used in tribal medicine. The leaves and stem bark of the plant were chosen for the study.

The *Pterocarpus indicus* stem bark and leaves were collected freshly from Senthankudi Village, Pudukkottai District, Tamil Nadu, India during December 2018. The plant was identified, authenticated (Voucher Specimen Number: 2952) and deposited in Department of Botany St. Joseph's College, Trichy.

The stem bark and leaves of *Pterocarpus indicus* revealed the presence of various phytochemical compounds viz., tannin, phlobatannin, saponin, flavonoids, steroids, terpenoids, anthocyanin, anthraquinone, coumarin, phenol, cardiac glycoside, xanthoprotein, alkaloids, emodin and carbohydrates. *Pterocarpus indicus* stem bark and leaves mediated silver nanoparticles were synthesized by mixing the stem bark and leaves extract with silver nitrate separately. Biosynthesized *Pterocarpus indicus* stem bark and leaves mediated silver nanoparticles were confirmed by analysing the excitation of surface plasmon resonance using UV- Vis spectrophotometer and the peak was observed at 439 nm for the stem bark and 435 nm for the leaves.

The FTIR spectrum illustrated that the stem bark-AgNPs shows the strong band primary amine at 3441.16 $cm^{-1}$ corresponds to 3441.16 $cm^{-1}$ corresponds to broad O-H stretching alcohol. The Strong band Isothiocyanate at 2077.79 $cm^{-1}$ corresponds to N=C=S stretching vibrations. The Medium band Alkene at 1634.81 $cm^{-1}$ corresponds to C=C stretching conjugated alkene and amine at 1405.54 $cm^{-1}$ corresponds to O-H bending alcohol.

The Strong band Alcohol at 1114.45 $cm^{-1}$ corresponds to C=O secondary alcohol, Anhydride at 1030.7 $cm^{-1}$ corresponds to CO-O-CO anhydride and Halo compound at

662.74 $cm^{-1}$ corresponds to C-Br stretching vibrations. The leaves-AgNPs shows the medium band primary amine at 3438.11 $cm^{-1}$ corresponds to N-H stretching vibrations. The strong band Isothiocyanate at 2085.17 $cm^{-1}$ corresponds to N=C=S stretching vibrations. The medium band Alkene at 1637.86 $cm^{-1}$ corresponds to C=C stretching vibrations, Amine at 1109.08 $cm^{-1}$ corresponds to C-N stretching vibrations. The Strong band Halo compound at 670.15 $cm^{-1}$ corresponds to C-Br stretching vibrations. Analysis of these spectra strongly suggested the presence of flavonoids and phenols, which were mainly responsible for the formation of silver nanoparticles by reducing silver nitrate.

The XRD pattern for silver nanoparticles synthesized using *Pterocarpus indicus* bark extract showed the characteristic peaks 38.7965, 44.7206, 65.0686 and 78.2134 in the 2Ɵ range. The diffraction peaks were obtained by leaves-AgNPs is observed at 38.4024, 46.3896, 65.1721 and 78.568 in the 2Ɵ range. The pattern (111), (200), (220) and (311) observed was face cantered cubic structure for silver according to (JCPDS, File No. 04-0783). SEM images of the synthesized silver nanoparticles lies between 37.9-124.5 nm region in case of leaves-AgNPs and 98.70-126 nm in case of stem bark-AgNPs. The average size of the nanoparticles is ~ 200 nm, whereas the shapes were spherical and cubic.

The zeta potential value falls within the range of −20 to −30 mV is considered as moderately stable, which clearly indicated that the synthesized S-AgNPs was moderately stable in nature. From the average particle size and PDI value it is found that produced nanoparticles are monodispersed in nature.

The synthesized silver nanoparticles of the various concentrations (20-100 µg/ml) exhibited potent alpha amylase inhibitory activity in a dose dependent manner. *Pterocarpus indicus* extracts showed inhibitory activity from 56.95% to 67.75% for

leaves and for the stem 65.95% to 80.36% at a concentration 100 µg/ml. Acarbose is a standard drug and showed the inhibitory activity from 70.27% to 82.88% at a concentration 100 µg/ml has been depicted.

The synthesized silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* showed the antidiabetic activity using alpha glucosidase inhibitory activity from 58.74% to 72.85% for leaves and from 48.83% to 64.64% for stem bark at a concentration 100 µg/ml. Acarbose is a standard drug at a concentration of (20-100 µg/ml) showed α-glucosidase inhibitory activity from 70.270% to 82.882% at the same concentrations of 100 µg/ml. The *in vitro* cytotoxicity study of the biosynthesized silver nanoparticles using leaves and barks of *Pterocarpus indicus* separately were investigated by an MTT assay against MCF-7 breast cancer cell line with different concentration 10-500 µg/ml to determine the IC50 (50% growth inhibition). The inhibitory concentration (IC50) value of biosynthesized silver nanoparticles using leaves is 54.60 µg/ml and for the stem barks-AgNPs is 126.4 µg/ml respectively. The stem barks- AgNPs was found more cytotoxic and also showed maximal antiproliferative effect as compared to the leaves-AgNPs on MCF-7 breast cancer cell lines.

The silver nanoparticles synthesized using a *Pterocarpus indicus* bark and leaf sample showed potent free radical scavenging activity. The synthesized silver nanoparticles showed 70.149% (leaves-AgNPs) and 75.929 (stem bark-AgNPs) activity at concentration 100 µg/ml while ascorbic acid gave 94.69 % at the same concentration in DPPH radical scavenging method.

The reducing power of ethanolic extracts of synthesized silver nanoparticles using leaves and bark of *Pterocarpus indicus* was performed and compared with standard ascorbic acid. Reducing power activity of stem bark-AgNPs (78.37%) and leaves-AgNPs

extracts (79.27%) as we compared to the standard drug ascorbic acid (84.68%) at a concentration 100 µg/ml.

The chromatogram of GCMS analysis revealed the presence of active constituents present in the extracts. So, in the present study, 10 bioactive compounds were identified from the ethanolic extracts of leaves and 20 bio active compounds from the stem barks of *Pterocarpus indicus*.

Docking studies by Maestro (Schrodinger) showed that the compounds cytidine (CAS) cyd had the highest docking score (-7.998) followed by 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon (-7.222), benzoldicar bonsaeure (-6.093), 1,2-benzenedicarboxylic acid, bis (2-ethylhexy) (-5.689) against alpha amylase enzymes acarbose which is -14.339 KJ/MOL which may be a potent anti-diabetic compounds because the high docking score, the compound cytidine (CAS) cyd will be potent antidiabetic drugs against alpha amylase activity. The compounds 2-(4-hydroxyl-2-butenyl)-2-nitrocyclo heptanon (-7.496) followed by trans-2-phenyl-1,3-dioxolane-4 (-6.472), cytidine (CAS) cyd (-6.233), phthalic acid, butyl ester, ester with butyl glyc (-5.036) against alpha glucosidase enzymes acarbose which is -14.452 KJ/MOL which may be a potent anti-diabetic compound because the high docking score, the compound 2-(4-hydroxyl-2-butenyl)-2-nitrocyclo heptanon will be potent antidiabetic drugs against alpha glucosidase activity.

Grid based docking study was used to analysis the binding modes of molecules with the amino acids present in the active site of the protein in order to study the interaction of the compounds with the estrogen receptor alpha (ER-α).

We also performed glide docking analysis by Schrodinger, where compared to all the compounds phthalic acid, butyl ester, ester with butyl glyc (-8.827) followed by

cytidine (CAS) cyd (-8.781), Bi-1,3-5-cyclo heptatrien-1-yl (CAS) (-8.353) showed the best docking score due to the strong binding bond between 3 ERT and compounds in favourable conformation. Tamoxifen (-12.982) is an antagonist of ER-alpha used to control the breast cancer and also it binds the amino acids and blocks the function of estrogen receptor and inhibits the function of human estrogen receptor.

The pharmacokinetic properties like GI absorption, Skin permeability, substrate or inhibitor of P- glycoprotein, blood brain barrier (BBB), Cytochrome P450 model used for substrate CYP2D6 and CYP3A4, inhibitor CYP1A2, CYP2C19, CYP2C9, bioavailability etc, were observed and the compounds like Palmitic acid, Nonadecanoic acid, Eicosatetrayonic acid and oleic acid shows good ADME properties. HOMO – LUMO gap reflect the chemical activity of the molecule. Compounds like Benzoldicarbonsaeure (-0.07166), 9-Octadecenoic acid (Z)- (CAS) Oleic acid (-0.09856), Hexadecanoic acid (CAS) Palmitic acid (-0.12114), Dodecanoic acid (CAS) Lauric acid (-0.14372) shows lowest HLG (eV) value.

To conclude, the results of above findings strongly prove that both leaves and stem bark of *Pterocarpus indicus* contain many important and potent phytochemicals. Further, the plant leaves and stem bark mediated green synthesized silver nanoparticles are proved to be effective antioxidants, antidiabetic and anticancer agents. In addition, outcomes of this work shows that most of the phytocompounds present in the plant leaves and stem bark have greater intestinal absorption, lower skin permeability, good lipophilicity and bioavailability. Also, the result showed that the distribution volume of the compound act as substrate and inhibitor for CYP3A4, this indicates that it can be metabolized in liver.

From the *in-silico* studies it is clearly showed that most of the bioactive compounds have a good binding energy. The present study revealed that the ethanolic stem bark and leaves extract of *Pterocarpus indicus* found to be pharmaceutical important plant, which is traditionally used for the treatment of various ailments and its medicinal application can be easily noticed by the presence of different bioactive molecules. In addition, further research is needed to identify and purify the active compounds responsible for therapeutic activity.

# 7. Perspectives on the Future Directions

In today's era nanotechnology is found to be very hopeful technology because of the size and property of dispersion of nanoparticles. Nanomedicine and nano devices market are growing rapidly. At the moment it is costly, so to brought down the cost of production lot of technological work is needed.

Development in technology may have lot of scope of nanomedicine in near future. Nanotechnology and nanoscience could make appropriate use of the herbal plants. The main approaches of nanotechnology and nanoscience are particle design and formulation are beginning to expand the market for many drugs.

In order to reduce or replace animal test with non-animal alternatives *in vitro* and *in silico* assays were used in the development of product and assessing the safety. Research into the use of small molecules as a drug is the key for the development of computer aided drug design. This is used to find out the activities of molecule and target interaction. Drug molecule has to fulfil, from the affinity to targets to minimal side effects while having adequate absorption, distribution, metabolism and excretion (ADME) properties.

Nature has blessed us with enormous wealth of herbal plants which are widely distributed all over the world as a source of therapeutic agents for the prevention and cure of various diseases. According to WHO, world's 80% population uses herbal medicines for their primary health care needs.

Epidemiologic studies indicates that diabetic patients are at significantly higher risk of common cancers. There is increasing evidence that breast cancer is associated with diabetes and mortality due to breast cancer is increased among women.

The present research work mainly focused on the medicinal properties of *Pterocarpus indicus* and investigated the inhibitory effects of silver nanoparticles synthesized using ethanolic stem bark and leaves extract of *Pterocarpus indicus*.

The outcomes of this research work are expected to bring depth to study antioxidants, antidiabetic and anticancer activity of leaves and stem bark of *Pterocarpus indicus* and also shows that the plant *Pterocarpus indicus* is found to be very important pharmaceutical plant with various bioactive compounds that can be useful for therapeutic activity.

This is an attempt to search for alternative drugs from medicinal plants with increased potency and lesser adverse effects than existing drugs. So, in future further research is needed to identify and purify the active compounds from *Pterocarpus indicus* that are responsible for antioxidants, antidiabetic and anticancer activity.

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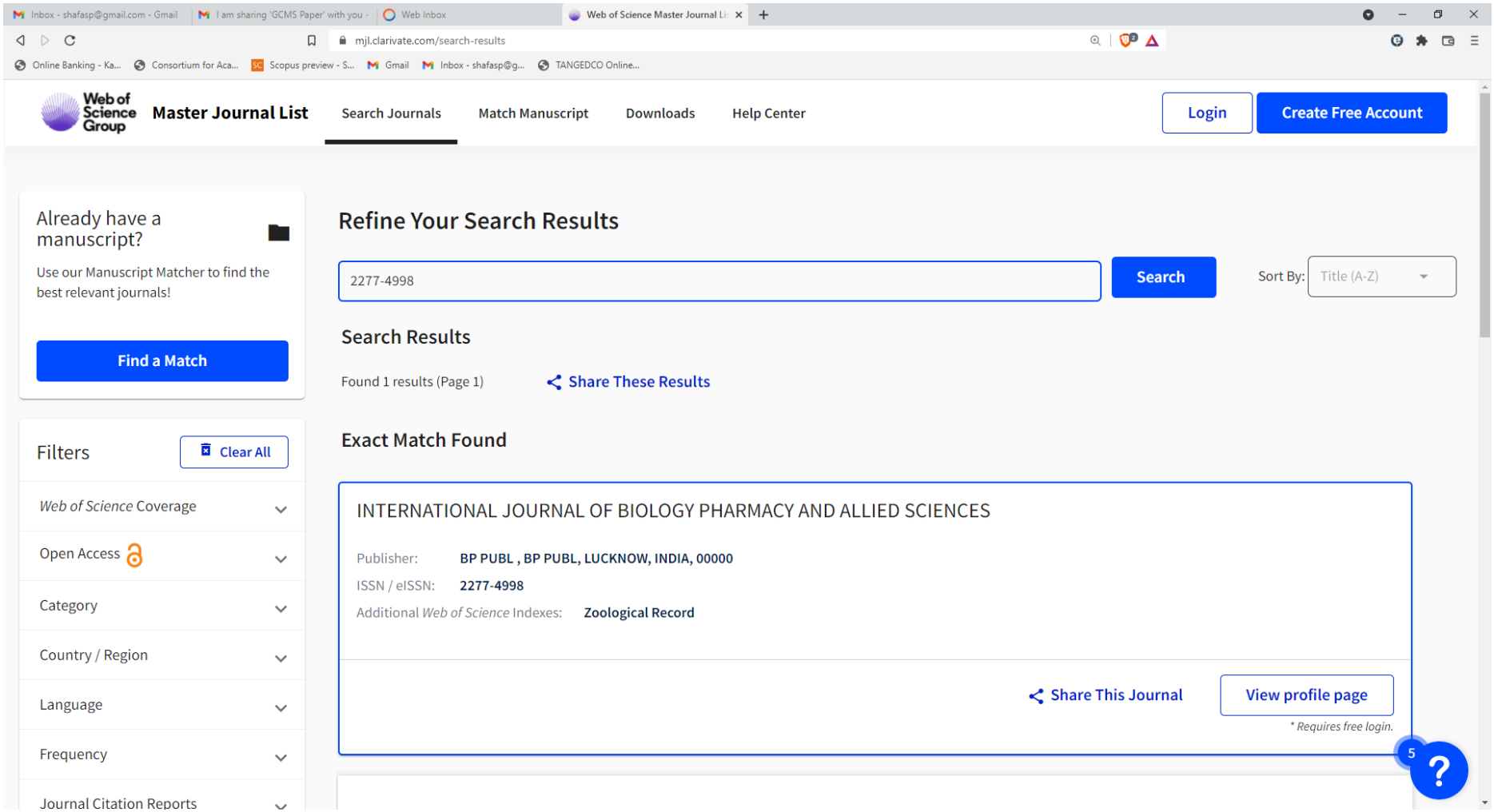
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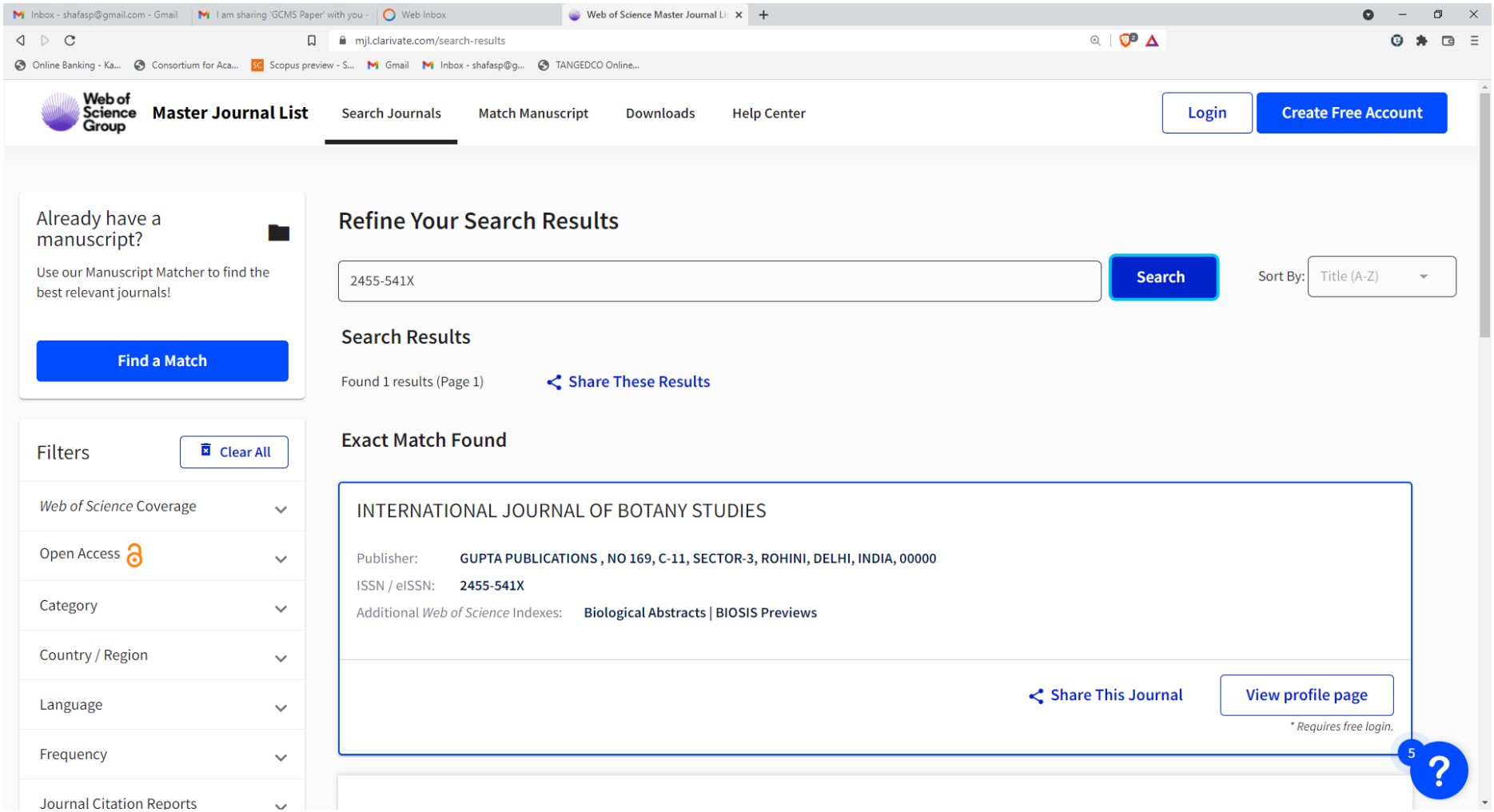
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# ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES USING LEAVES AND STEM BARK OF *PTEROCARPUS INDICUS* WILLD

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## ABSTRACT

In the present study, synthesis of silver nanoparticles (AgNPs) using leaves and stem bark of *Pterocarpus indicus* were compared by studied for alpha amylase, alpha glucosidase inhibition assay and also examined for its antioxidant activities by using free radical 1,1 diphenyl -2 picrycl hydrozyl DPPH scavenging method under *in vitro* model separately. These synthesized silver nanoparticles were characterized using UV-visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM), X-ray Diffraction (XRD) and zeta potential for the further confirmation. The synthesized silver nanoparticles were checked with the colour variation and it was confirmed by UV-vis spectral analysis. The morphology of the synthesized nanoparticles was analyzed using SEM. The XRD was done to find out the crystalline structure of the compound. FTIR measurements are carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the silver NPs synthesized using *Pterocarpus indicus*. The result revealed that the leaves-AgNPs

showed significant inhibitory effect on alpha amylase (77.1%) and for the stem bark-AgNPs (74.2%). Alpha glucosidase showed inhibitory effect on leaves-AgNPs (77.5%) and for stem bark-AgNPs (75.7%) were compared with standard acarbose drug. The antioxidant activities by DPPH method showed activity for leaves-AgNPs (70.15%), and for the stem bark-AgNPs (75.92%) were compared with standard ascorbic acid by measuring the percentage inhibitory effect at the concentration of 100µg/ ml respectively.

Maximum inhibition was observed for reducing power assay at (79.27 %) for stem bark and for leaves (78.37 %) and compared to the standard drug ascorbic acid (84.68 %) at a concentration of 100 µg/ml respectively. Therefore, it is suggested that the synthesized silver nanoparticles (AgNPs) using leaves and stem bark of *Pterocarpus indicus*is a potential source for natural antidiabetic and antioxidant compounds and could have potential use in the management of diabetes mellitus.

**Keywords: leaves-AgNPs and stem bark-AgNPs of *Pterocarpus indicus*, alpha amylase inhibitory activity, alpha glucosidase inhibitory activity, antioxidant (DPPH) activities**

## INTRODUCTION

Nanotechnology is a rapidly growing in the field of science for synthesizing and utilizing nano-sized particles **[1]**. For the synthesis of silver nanoparticles, such as thermal decom-position, electrochemical, micro-wave assisted process and green chemistry methods are available **[2]**. Many of the nanoparticle synthesis or production methods of nanoparticles involve the use of hazardous chemicals, low material conversions and high energy requirements **[3]**. So, there is need to develop an eco-friendly techniques and methods for nanoparticle synthesis without using any toxic chemicals. Thus, synthesising silver nanoparticles from plant extracts are gaining importance and also are considered as a simple, economic and viable alternative to chemical synthetic procedure **[4]**. Silver nanoparticles reduces toxicity and is involved in lowering of blood glucose level, higher serum insulin, higher glucokinase activity and boost up immune system, reduces kidney crystal formation and higher the expression of insulin level **[5]**, Silver nanoparticles used in wide range of application such as antimicrobial**,** anti-inflammatory**,** antiviral and anti- diabetic and also involved in the prevention of diabetic wound healing (ointments) **[6]**.

Diabetes mellitus is a universal endocrine metabolic disorder that affects people in both developed and developing countries. Due to lack of insulin secretion and action, leads to the metabolic changes of carbohydrates, proteins, fats and lipids **[7]**. It is designated by hyperglycemic condition (high blood sugar levels), because the pancreas do not produce enough insulin/ cells do not respond for the production of insulin **[8]**. Genetic factors and lifestyle may be the causes of diabetes **[9]**.

Type-1 diabetes mellitus occurs due to the deficiency of insulin secretion which is produced by β-cells of pancreas. Type-2 diabetes mellitus is caused by insulin resistance/β-cell dysfunction **[10]**. The treatment for this disorder is insulin injection and antidiabetic drug therapy. α -amylase and α- glucosidase are the two important digestive enzymes involved in the breakdown of carbohydrates and helps in intestinal absorption. α- amylase is responsible for the breakdown of long chain carbohydrates and α- glucosidase results in breakdown of starch and disaccharides into glucose **[11]**. α -amylase and α- glucosidase inhibitors are useful for lowering the process of glucose absorption and decreases glucose level in blood **[12]**. During hyperglycemic condition, reactive oxygen species (ROS) gets generated and leads to lipid-peroxidation, membrane damages and production of secondary complications such as kidney, eye, blood vessel and nerve damage **[13]**. Antioxidant plays a vital role in the inhibition of lipid peroxidation radical chain reaction, scavenging of free radicals, active oxygen species by rising a reaction cycle and to chelate heavy metal ions **[14]**.

*Pterocarpus indicus* belongs to the family Fabaceae popularly known as vengai maram and it's a medicinally valuable species widely distributed in the region of tropical and subtropical south Asia as Malaysia, Philippines, Brunei, Thailand, and Indonesia **[15]**. In the ayurvedic Pharmacopoeia of India, *Pterocarpus indicus*used in the treatment of krmiroga (worm infection), kustha (leprosy), prameha (diabetes), pandu (anemia), and medodosa (obesity), strong antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, and anticancer activities and is used for the treatment of diabetes, jaundice, ulcer, gastritis **[16]**. Their phytochemical studiescontain iso-flavonoids, terpenoids and related phenolic compounds, ß-sitosterol, lupenol, epicatechin and glycosides **[17,18]**.

Therefore, this study was designed to synthesize AgNPs using leaves and stem bark of *Pterocarpus indicus* and to evaluate for *in vitro* antioxidant and antidiabetic activity separately.

**MATERIALS AND METHODS:**

**Collection of Plant Material:**

The *Pterocarpus indicus* bark and leaves were collected in the month of December from the Senthankudi Village, Pudukkottai, Tamil Nadu, India. The plant was identified and authenticated by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirappalli, Tamil Nadu for identifying the plants.

**Chemicals and Reagents:**

Alpha (α)-Glucosidase, porcine pancreas alpha(α)-amylase, p-nitrophenyl-α-D-glucopyranose (p-NPG), 3,5-dinitrosalicylic acid (DNS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and acarbose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Soluble starch, sodium potassium tartarate, sodium dihydrogen phosphate (NaH2PO4), Di-sodium hydrogen phosphate (Na2HPO4) sodium chloride, sodium hydroxide, potassium ferricyanide, ferric chloride (FeCl3) were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

**Instruments:**

Lambda 35, Perkin Elmer Spectrophotometer, Malvern zetasizer version 2.2., XPERT-PRO Machine and TEM, JEOL-JEM 2100

**Ethanolic extract of plant preparation:**

The *Pterocarpus indicus* leaves and stem barks were collected and made into small pieces. The collected plant parts were thoroughly washed with tap water and air dried in a shadow that is free from sunlight till it becomes dried nicely. Then it is crushed in an electrical grinder and the powder was separated, which were stored individually in air-tight containers and kept in a cool, dark and dry place for further study. The ethanol extract was prepared by taking 20g of the powdered sample each and it is soaked in 40ml ofethanol for 24hours. Then it was extracted using hot percolation method. The extract was filtered using whatman No.41 filter paper in a clean beaker and used for the further study **[19]**.

**Optimization and Synthesis of Silver Nanoparticles:**

1mM silver nitrate was prepared in a 50 ml standard flask. 2.5 ml of the ethanolic extract of each leaves and stem bark of *Pterocarpus indicus*sample (25µl, 50µl, 75µl

and 100µl) was mixed with 1mM of silver nitrate solution without any contamination with continuous and constant stirring which react at an ambient condition and Ag get reduced in to Ag+ ion.

The colour change was observed for the reaction mixture from transparent white to dark brown indicates the formation of silver nitrate. The presence of reduction of Ag+ ion was confirmed overtime by the UV-Spectral analysis **[20]**.

**Characterization Techniques:**

Characterization of synthesized nanoparticles were carried out to learn the characteristic wavelength and functional group bound to silver nanoparticles by UV-Vis spectra and FTIR and its size, crystalline nature and elemental composition using SEM, XRD and zeta potential **[21, 22]**.

**UV-Visible Analysis:**

The optical properties of silver nanoparticles were characterized using UV-Vis spectrophotometer. Silver nitrate was added to the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* separately, the colour change was observed for the reaction mixture from transparent white to dark brown indicates the formation of silver nitrate. UV was taken after 24 hours of addition. The absorbance was recorded between 350-500nm

**FTIR analysis:**

Fourier Transform Infrared Spectroscopy is otherwise called as FTIR Analysis or FTIR Spectroscopy. The synthesized nanoparticle can be scanned by infrared light and chemical properties like organic, polymeric and inorganic materials were observed by this method. Fourier Transform Spectrometer absorbs infrared spectra within the range of 400-4500$cm^{-1}$. At a particular frequency, multiple functional groups may be absorbed and it gives rise to different characteristic absorptions.

**XRD:**

X-ray diffraction (XRD) analysis is used to study the nanomaterials (with structural features in the range of 1-100 nm). The structure of nanomaterials has been probed by XRD method.The position of values of product (crystallinity or amorphous nature) can be identified by this technique. With, respect to d-spacingvalues; the fingerprint regions of relative intensity are found in XRD analysis.

**SEM Analysis:**

SEM analysis of synthesized silver nanoparticles were performed to evaluate the surface morphology of nanoparticles. Silver nanoparticles were prepared and dried well to remove the moisture content and images

were taken by using FEI Quanta 250 FEG SEM operating at 10 kV.

**Zeta Potentiometer:**

The zeta potential was measured by using Zeta Sizer (Malvern Instruments) having zeta cells, polycarbonate cell with gold-plated electrodes and using water as medium for sample preparation. Zeta potential determines the surface potential of silver nanoparticles and it is essential for the characterization of stability of nanoparticles.

**Antioxidant activity (DPPH free radical scavenging activity) determination**

The antioxidant activity of the synthesized silver nanoparticles of leaves and stem bark of *Pterocarpus indicus* were examined on the basis of the scavenging effect on the stable DPPH free radical activity **[23]**. 300 µl of ethanolic solution of DPPH (0.05 mM) was added to 40 µl of each leaves-AgNPs and stem bark-AgNPs with different concentrations of 20 - 100 µg/ml separately and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectro-photometrically at 540 nm. Ethanolwas used to set the absorbance zero. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation **[24]**.

Percent (%) inhibition of DPPH activity = [(A – B) / A] x 100

Where B and A are the absorbance values of the test and of the blank sample, respectively.

**MEASUREMENT OF REDUCING POWER**

The ethanolic extracts of leaves and bark of *Pterocarpus indicus* were taken in different concentrations in phosphate buffer (0.2 mol /L, pH 6.6) and incubated with potassium ferricyanide (1 g /100 mL water) at 50$^{o}$C for 20 min. the reaction was terminated by adding TCA solution (10 g /100 mL water), centrifuged at 3000 rpm for 10 min and the supernatant was mixed with ferric chloride (0.1 g/100 Ml water), the absorbance measuredat 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

Percent (%) inhibition of DPPH activity = [(A – B) / A] x 100Where B and A are the absorbance values of the test and of the blank sample, respectively **[24, 25]**.

**Alpha-Amylase Inhibitory Assay**

A total of 250$\mu$L of synthesized silver nanoparticles of leaves and stem bark of *Pterocarpus indicus* (20-100 µg/ml) was placed in a tube separately and 250 $\mu$L of 0.02M sodium phosphate buffer (pH 6.9) containing $\alpha$-amylase solution (0.5mg/mL) was added. This solution was preincubated at

25ºC for 10 min, after which 250 $\mu$L of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at for 25ºC for 10min. The reaction was terminated by adding 500 $\mu$L of dinitro salicylic acid reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water **[25]**.

The $\alpha$-amylase inhibitory activity was calculated as percentage inhibition:

**% Inhibition = [(Abs control – Abs extract) / Abs control] x 100**

**Alpha-Glucosidase Inhibitory Assay**

The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 $\mu$L of $\alpha$- glucosidase (1.0U/mL) was preincubated with 50 $\mu$L of the different concentrations 20-100 µg/ml of the synthesized silver nanoparticles of leaves and stem bark of *Pterocarpus indicus*for 10min separately. Then 50 $\mu$L of 3.0mM (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37ºC for 20min and stopped by adding 2mL of 0.1M $Na_2CO_3$. The $\alpha$-glucosidase activity was determined by measuring the yellow-colouredpara nitrophenol released from pNPG at 540 nm. The results were expressed as percentage of the blank control **[26]**.

The $\alpha$-glucosidase inhibitory activity was calculated as percentage inhibition:

**%Inhibition = [(Abs control – Abs extract) / Abs control] x 100**

**Statistical Analysis**

All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at $P < 0.05$ were considered to be significant.

**RESULTS AND DISCUSSION**

**Visual colour change and UV-Vis spectroscopy:**

The presence of silver nanoparticle was analysed by using UV-Vis spectral technique at room temperature. The gradual change in the colour of samples from colourless to dark brown colour was observed and the bioreduction of Ag+ in the solvent extract. The UV visible absorption spectrum was noted at the range of 435 nm for the leaves-AgNPs and at the range of 439 nm for the stem bark-AgNPs **(Figure 1)**. The previous studies suggested that the silver nanoparticles are known to exhibit UV-Visible absorption in the range of 400-500 nm. The sharp absorption

bands of *Pterocarpus marsupium* silver nanoparticles were observed around 431 nm **[27]**.

**Functional group determination using FT-IR spectroscopy:**

The FT-IR spectrum of synthesized gives details about the functional group involved in the silver ions reduction **(Figure 2)**. The FT-IR spectra ofsynthesized silver nanoparticles by using *Pterocarpus indicus* leaf and barkextract are shown in (**Tables 1 and 2)**. Some of the peaks appeared in the FT-IR spectrum of stem bark-AgNPs **(Figure 2b)**, which were disappeared in FT-IR spectra of leaves-AgNPs **(Figure 2a)**. This disappearance of peaks is due to phytochemicals present in the bark extract, involves silver nanoparticles reduction. Analysis of these spectra strongly suggested the presence of flavonoids and phenols, which were mainly responsible for the formation of silver nanoparticles by reducing silver nitrate.From the FTIR spectral analysis it is concluded thathydroxyl and carboxyl groups present may act asreducing and stabilizing agent and phenolic grouppresent may act as capping agent **[28, 29]**. The previous studies reported that the main components such as steroids, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides, and glycerides present in the leaf extract of *P. santalinus* are prime responsible for the observed reduction and capping during the synthesis of Ag NPs **[30]**.

**X-ray diffraction (XRD):**

The crystalline nature of silver nanoparticles was confirmed using X-ray crystallography. The X-ray diffractogram pattern of synthesized silver nanoparticles was represented in **Figure 3**. The diffraction peaks were obtained by leaves-AgNPs is observed at 38.4024, 46.3896, 65.1721 and 78.568 in the 2Ɵ range **(Figure 3a)**. The obtained XRD pattern for silver nanoparticles synthesized using *Pterocarpus indicus* bark extract showed the characteristic peaks 38.7965, 44.7206, 65.0686 and 78.2134 in the 2Ɵ range **(Figure 3b)**.

The intense diffraction peak is obtained at 2Ɵ values 27.7794, 32.1627, 38.0824, 44.7077, 65.0407, 78.187 and 78.4575. The peak corresponds to 38.0824, 44.7077 following diffraction facets are (111), (200) respectively. This pattern (111),(200),(220) and (311) reflection of the face cantered cubic structure for silver according to (JCPDS, File No. 04-0783). Unassigned peaks are also present in the graph this may be due to extract contains some phytochemicals which may be capping the nanoparticles surface. The XRD pattern clearly showed that the Ag NPs formed by the reduction of Ag+ ions using *P. santalinus* leaf extract are crystalline in nature. Similar results

were reported for AgNPs in the literature **[31-33]**.

**SEM image:**

SEM images of the synthesized silver nanoparticles lie between 37.9-124.5 nm region in case of leaves-AgNPs **(Figure 4a)** and 98.70-126 nm in case of stem bark-AgNPs sample **(Figure 4b)**. SEM image reveals that most of the synthesized silver nanoparticles are nearly spherical in shape and cubic. The SEM image further ascertains that the silver nanoparticles are predominantly spherical in morphology with their sizes ranging from 20 to 300 nm was previously reported **[34]**. From the previous studies suggested that the SEM analysis it was found that *Pterocarpus marsupium* Roxb.silver nanoparticles have spherical shape and the assembling of silver nanoparticles on the surface **[35]**.

**Particle Size Distribution and Zeta Potential Studies:**

The average size of the nanoparticles, particle size distribution, and polydispersity index (PDI) of the synthesized silver nanoparticles was characterized using particle size analyzer.. The average particle size diameter of synthesized leaves silver nanoparticle is1842 nm with poly dispersity index of 0.247 **(Figure 5a)** and for the stem bark-AgNPs exhibited polydisperse mixture with the size ranging of 385 nm with poly dispersity index of 0.063 **(Figure 6a)**. From the average particle size and PDI value it is found that produced nanoparticles are monodispersed in nature.

Zeta potential is used for determining the stability of synthesized silver nanoparticles. For leaves-AgNPs zeta potential measured was found to be −13.1 mVwith peak area of 100% intensity (fig. 5b). The biosynthesized stem bark-AgNPs had a negative charge with a zeta potential value −16.3 mV **(Figure 6b)**. These values indicate the full stabilization of nanoparticles. The previous studies suggested that the particle size is analyzed by using zeta sizer and the average particle size was found to be 148.5 nm with polydispersity index 0.336 and intercept 0.963. Zeta potential measured was found to be -28 mV with peak area of 100 % intensity. These values indicate the stabilization of silver nanoparticles for the *Pterocarpus marsupium* silver nanoparticles **[36]**.

**Antioxidant activity of silver nanoparticles synthesized using leaves and stem bark of *Pterocarpus indicus*by DPPH method**

The result showed that thesilver nanoparticles synthesized using a *Pterocarpusindicus*bark and leaf sample of ethanolicextracthad better percentage antioxidant activities at high concentrations when compared with ascorbic acid **(Table 3)**. The synthesized silver nanoparticles showed

70.149% (leaves-AgNPs) and 75.929 (stem bark-AgNPs) activity at concentration 100 µg/ml while ascorbic acid gave 94.69 % at the same concentration. Methanol extract of *Pterocarpus marsupium* is found to possess highest DPPH radical scavenging activity followed by aqueous and ethyl acetate extracts **[37]**.

**Antioxidant activity of stem barks and leaves of *Pterocarpus indicus* by reducing power method:**

The reducing power of ethanolic extracts of leaves and bark of *Pterocarpus indicus* was performed and showed concentration dependent manner **(Figure 7)**. Ethanolic extract of stem bark of *Pterocarpus indicus* (78.37%) exhibits good reducing power activity among leaves extracts (79.27%) as we compared to the standard drug ascorbic acid (84.68%) at a concentration 100 µg/ml **(Table 4)**. It is believed that antioxidant activity and reducing power are related. Reductones inhibits LPO by donating a hydrogen atom and thereby terminating the free radical chain reaction.

***In vitro* alpha amylase inhibitory assay:**

In this study the *in vitro* alpha amylase inhibitory activities of the silver nanoparticles synthesized using a *Pterocarpus indicus*extract was investigated. The synthesized silver nanoparticles showed inhibitory activity from 65.954% to 80.369% for stem and 56.954%to 67.756% for leaves at concentration 100 µg/ml **(Table 5)**. Acarbose is a standard drug for α-amylase inhibitor. Acarbose at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 70.270% to 82.882% at the same concentrations 100 µg/ml. A comparison of α–amylase inhibitory activity between the standard drug has been depicted in **(Figure 8)**.

Our results are in accordance with the previous study wherein, some mechanisms of antidiabetic drugs, such as suppress hepatic glucose production (biguanides), stimulate insulin secretion (sulfonylureas and glinides), and absorption of intestinal carbohydrates to maintain postprandial glucose level (α-glucosidase and α-amylase inhibitors), improve the sensitivity of insulin receptor and peripheral glucose uptake (thiazolidinediones and metformin) or insulin**[33]**. The previous study suggested an *in vitro* alpha-amylase inhibition model wasused to screen the *Pterocarpus marsupium* Roxb. silver nanoparticles to evaluatethe hypoglycemic effects **[34]**. Strong *in vitro* inhibitory effects of *Pterocarpus marsupium* Roxb. latex was observed on α-amylase and α-glucosidase

with IC50 of 2.97 and 0.54 µg/ml respectively **[38]**. Aqueous extract of the PM latex had shown a marked α-glucosidase inhibitory activity **[39]**.

***In Vitro* α-glucosidase inhibitory assay:**

The results of antidiabetic activity using α- glucosidase inhibitory assay of the silver nanoparticles synthesized using a *Pterocarpus indicus* extract. The synthesized silver nanoparticles showed inhibitory activity from 48.837% to 64.648% for stem and 58.747%to 72.855% for leaves at concentration 100 µg/ml **(Table 6)**. Acarbose is a standard drug for α-amylase inhibitor. Acarbose at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 70.270% to 82.882%at the same concentrations 100 µg/ml **(Figure 9)**.

The previous study suggested, one therapeutic approach for treating diabetes is decreasing post-prandial hyperglycemia by delaying glucose absorption through carbohydrate-hydrolyzing enzymes inhibition, α-glucosidase, in the digestive tract. Inhibitors of these enzyme delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise. **[40, 41]**).

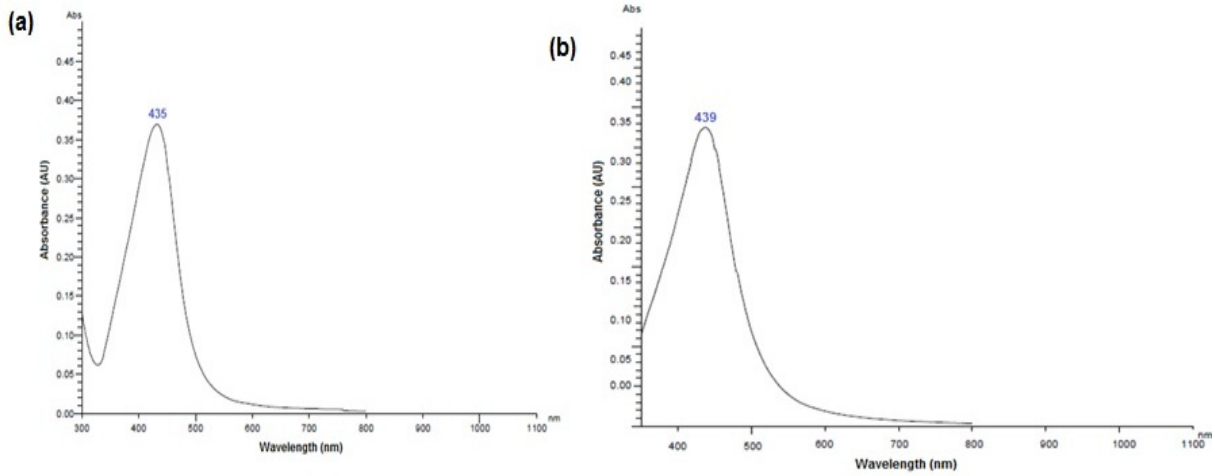



**Figure 1: UV-VIS spectrum of synthesized silver nanoparticles using *Pterocarpus indicus* (a) leaves- AgNPs (b) stem bark-AgNPs**

**Table 1: FTIR band values of synthesized silver nanoparticles using of leaves of *Pterocarpus indicus***

| Functional group | Band | Frequency. $Cm^{-1}$ |
|---|---|---|
| Primary amine | Medium band | 3438.11 $cm^{-1}$ corresponds to N-H stretching vibrations |
| Isothiocyanate | Strong band | 2085.17 $cm^{-1}$ corresponds to N=C=S stretching vibrations |
| Alkene | Medium band | 1637.86 $cm^{-1}$ corresponds to C=C stretching vibrations |
| Amine | Medium band | 1109.08 $cm^{-1}$ corresponds to C-N stretching vibrations |
| Halo compound | Strong band | 670.15 $cm^{-1}$ corresponds to C-Br stretching vibrations |

**Table 2: FTIR band values of synthesized silver nanoparticles using of stem bark of *Pterocarpus indicus***

| Functional group | Band | Frequency. $Cm^{-1}$ |
|---|---|---|
| Primary amine | Strong band | 3441.16 $cm^{-1}$ corresponds to broad O-H stretching alcohol |
| Isothiocyanate | Strong band | 2077.79 $cm^{-1}$ corresponds to N=C=S stretching vibrations |
| Alkene | Medium band | 1634.81 $cm^{-1}$ corresponds to C=C stretching conjugated alkene |
| Amine | Medium band | 1405.54 $cm^{-1}$ corresponds to O-H bending alcohol |
| Alcohol | Strong band | 1114.45 $cm^{-1}$ corresponds to C=O secondary alcohol |
| Anhydride | Strong band | 1030.7 $cm^{-1}$ corresponds to CO-O-CO anhydride |
| Halo compound | Strong band | 662.74 $cm^{-1}$ corresponds to C-Br stretching vibrations |

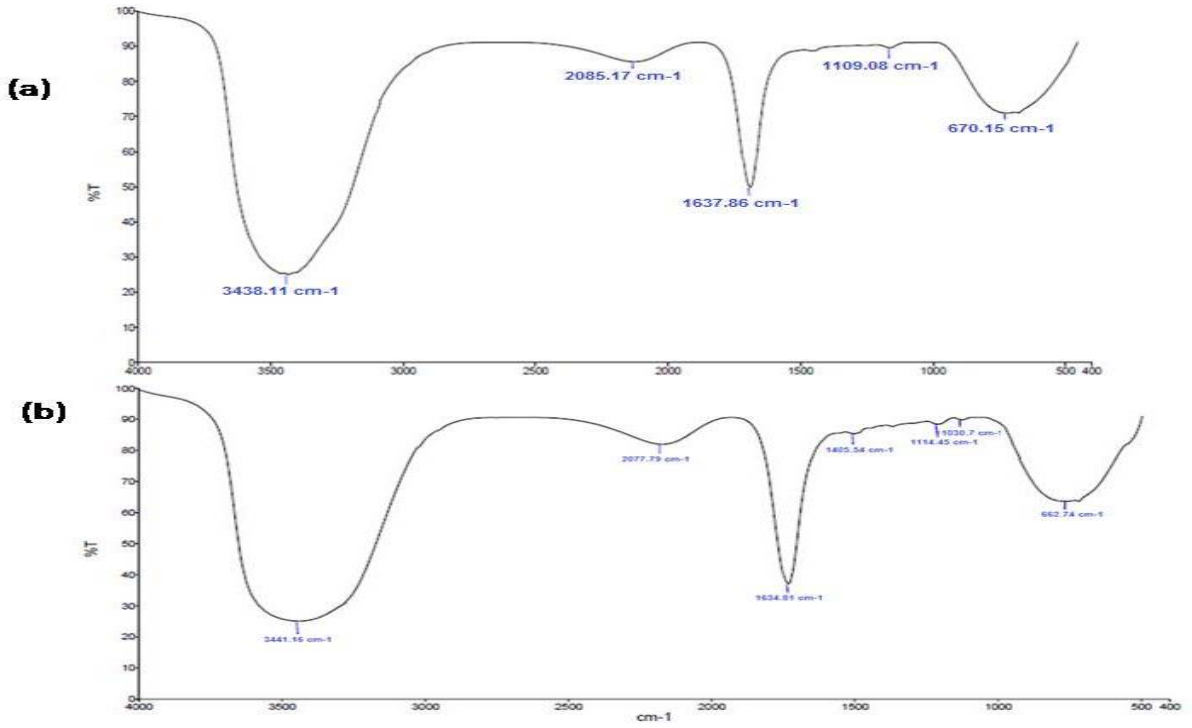



**Figure 2: FTIR spectrum of synthesized silver nanoparticles using *Pterocarpus indicus* (a) leaves-AgNPs (b) stem bark-AgNPs**

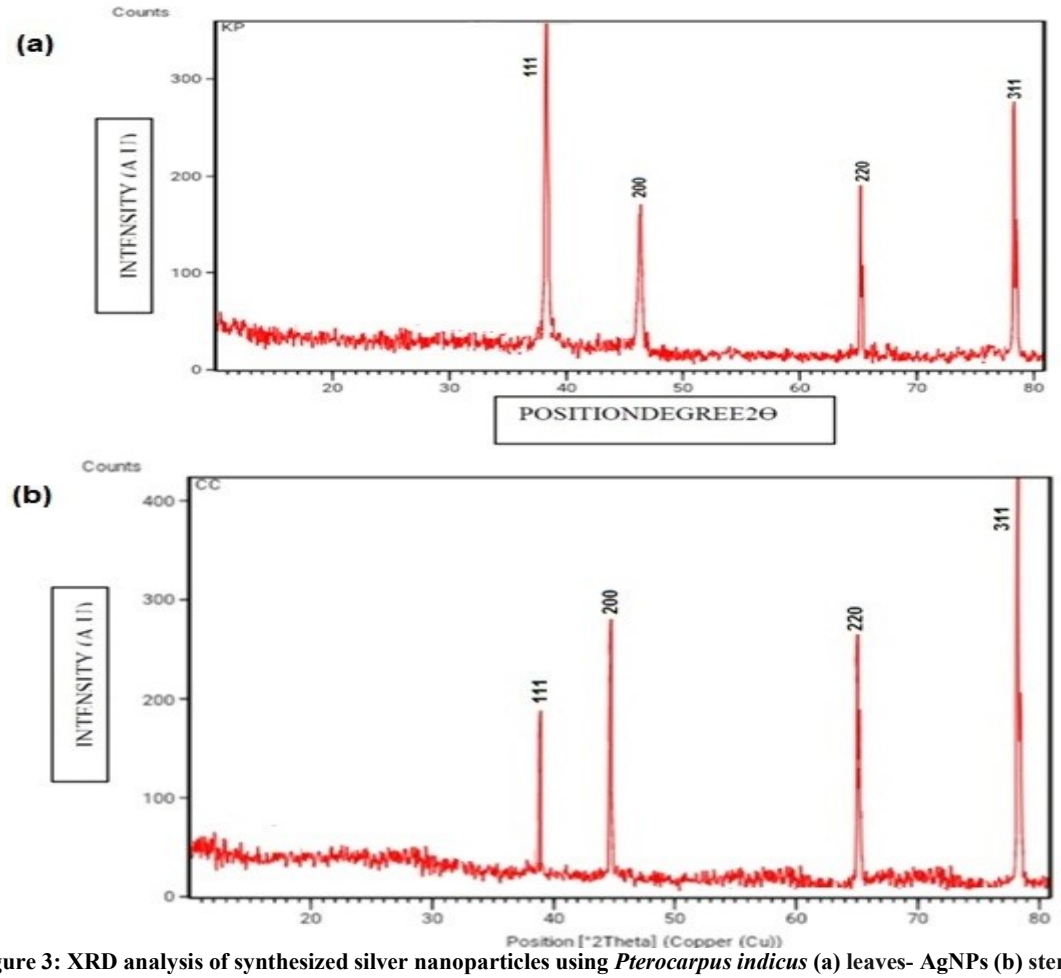



**Figure 3: XRD analysis of synthesized silver nanoparticles using *Pterocarpus indicus* (a) leaves- AgNPs (b) stem bark-AgNPs**

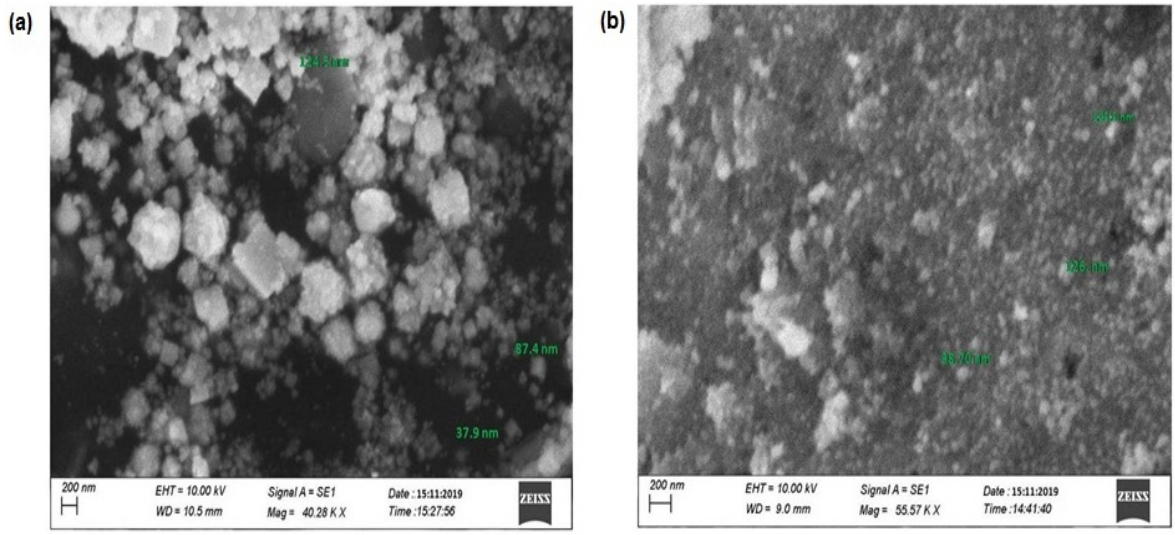
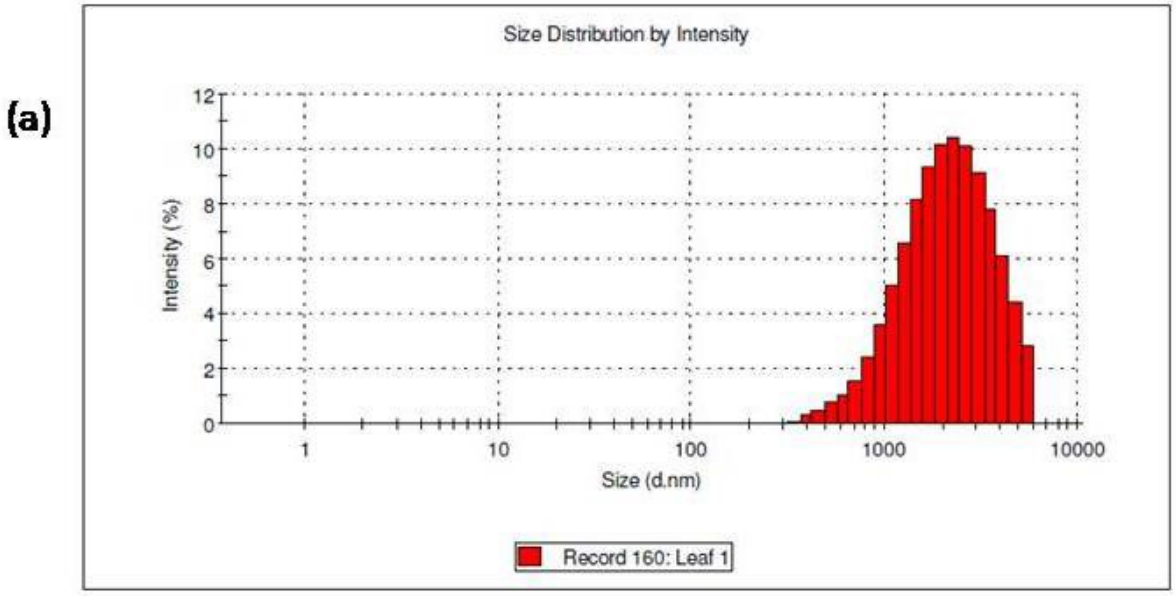



**Figure 4: SEM photograph of synthesized silver nanoparticles using *Pterocarpus indicus* (a) leaves- AgNPs (b) stem bark AgNPs**



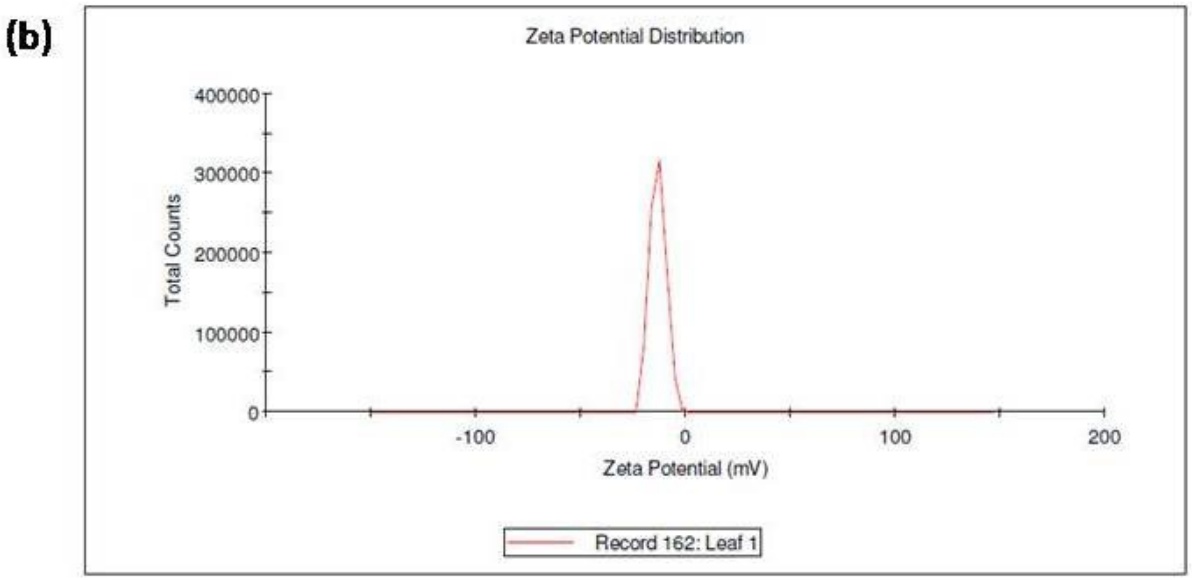




**Figure 5: (a) particle size distribution, and (b) zeta potential measurement of the biosynthesized AgNPs using *Pterocarpus indicus* leaves extract**

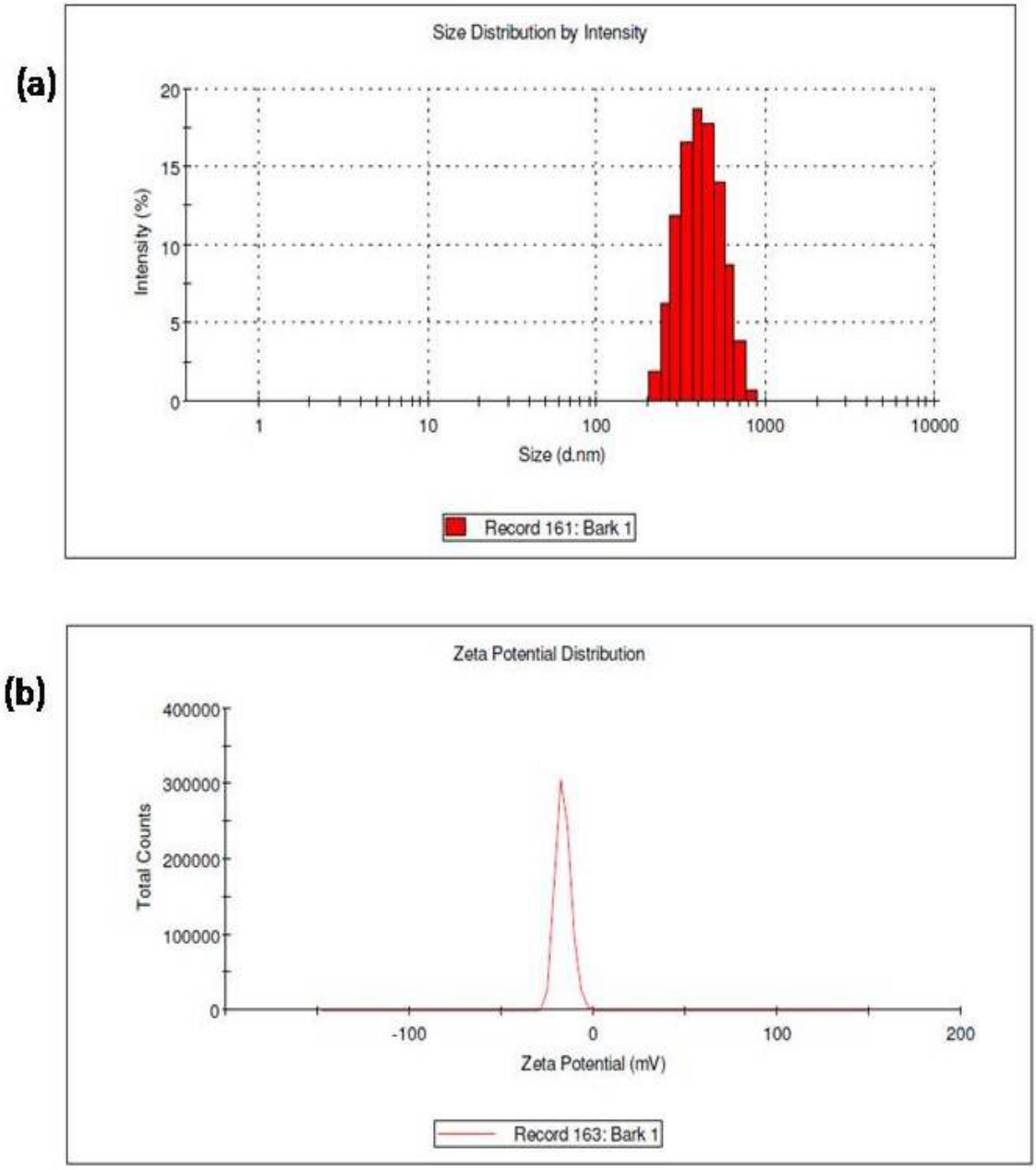



**Figure 6: (a) particle size distribution, and (b) zeta potential measurement of the biosynthesized AgNPs using *Pterocarpus indicus* stem bark extract.**

**Table 3: Antioxidant activity of silver nanoparticles synthesized using a *pterocarpus indicus* by DPPH activity**

| S. No. | Concentration | DPPH ACTIVITY% | | |
|---|---|---|---|---|
| | | Leaves-AgNPs | Stem bark-AgNPs | Acarbose |
| 1 | 20 (µg/ml) | 55.22±0.812 | 57.76±0.812 | 61.68±0.824 |
| 2 | 40 (µg/ml) | 57.46±0.824 | 62.98±0.808 | 72.85±0.824 |
| 3 | 60 (µg/ml) | 59.70±0.824 | 69.47±0.808 | 79.74±0.808 |
| 4 | 80 (µg/ml) | 64.17±0.824 | 71.46±0.824 | 82.34±0.824 |
| 5 | 100 (µg/ml) | 70.14±0.008 | 75.92±0.824 | 94.69±0.808 |

**At the 0.05 level, the population means are significantly different**

**Table 4: Antioxidant activity of stem barks and leaves of *Pterocarpus indicus*by reducing power activity**

| S. No. | Concentration | REDUCING POWER ACTIVITY % | | |
|---|---|---|---|---|
| | | Leaves-AgNPs | Stem bark-AgNPs | Acarbose |
| 1 | 20 (µg/ml) | 52.25±0.808 | 53.15±0.824 | 64.86±0.826 |
| 2 | 40 (µg/ml) | 56.75±0.824 | 61.26±0.824 | 66.66±0.824 |
| 3 | 60 (µg/ml) | 68.46±0.824 | 67.56±0.824 | 70.27±0.832 |
| 4 | 80 (µg/ml) | 73.81±0.824 | 75.67±0.808 | 81.08±0.828 |
| 5 | 100 (µg/ml) | 78.37±0.808 | 79.27±0.808 | 84.68±0.824 |

**At the 0.05 level, the population means are significantly different**

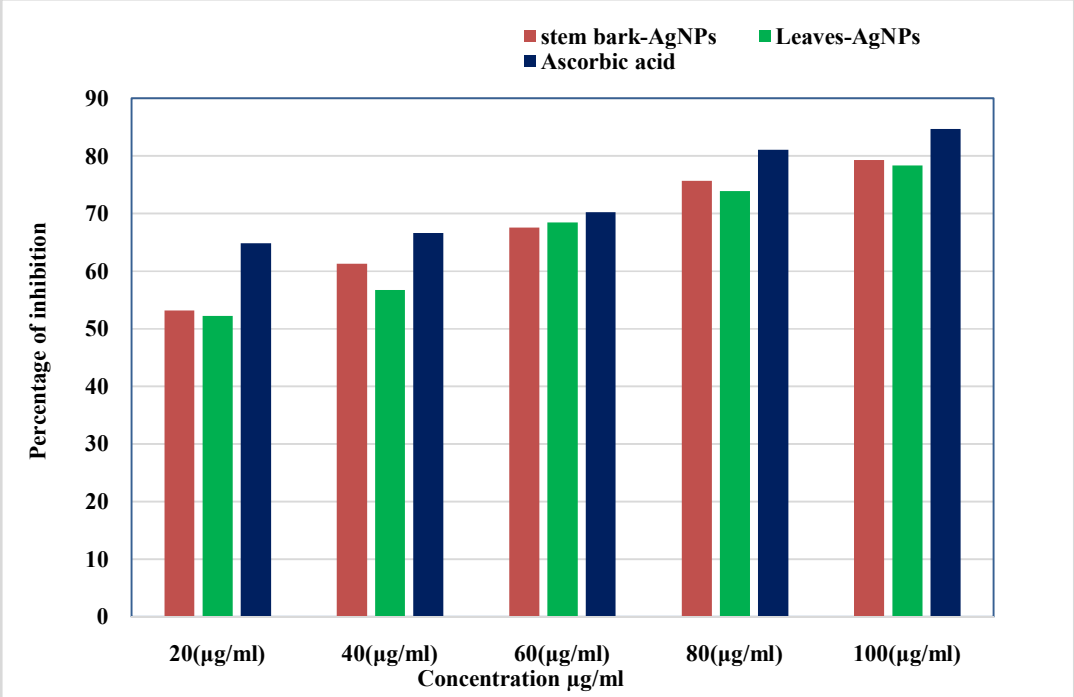



**Figure 7: Antioxidant activity of stem bark and leaves of *Pterocarpus indicus* by reducing power activity**

**Table 5: *In vitro* antidiabetic activity of the silver nanoparticles synthesized using a *Pterocarpus indicus* extract using alpha amylase method and comparison with standard drug acarbose**

| S. No. | Concentration | Alpha amylase (%) | | |
|---|---|---|---|---|
| | | Leaves-AgNPs | Stem bark-AgNPs | Acarbose |
| 1 | 20 (µg/ml) | 56.04±0.832 | 65.95±0.832 | 70.27±0.824 |
| 2 | 40 (µg/ml) | 59.64±0.824 | 67.75±0.824 | 74.77±0.824 |
| 3 | 60 (µg/ml) | 60.54±0.832 | 73.16±0.832 | 80.18±0.824 |
| 4 | 80 (µg/ml) | 65.95±0.824 | 78.56±0.824 | 81.98±0.824 |
| 5 | 100 (µg/ml) | 67.75±0.824 | 80.36±0.824 | 82.88±0.824 |

**At the 0.05 level, the population means are significantly different**

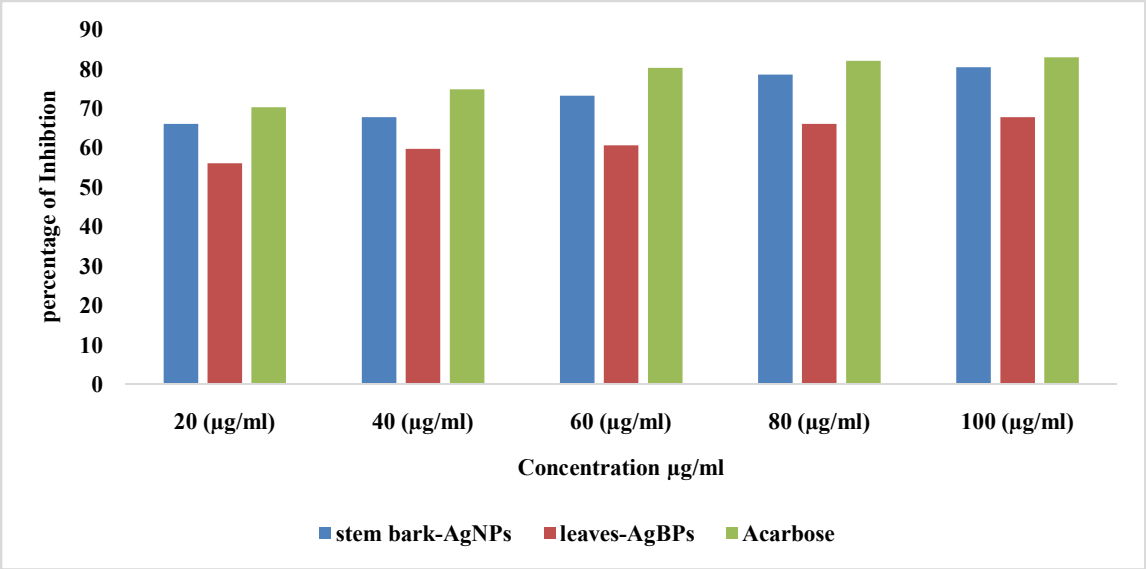



**Figure 8: α-Amylase inhibitory activity of Acarbose vs silver nanoparticles synthesized using a *Pterocarpus indicus***

**Table 6: *In vitro* antidiabetic activity of the synthesized silver nanoparticles using alpha glucosidase method and comparison with standard drug acarbose**

| S. No. | Concentration | Alpha Glucosidase (%) | | |
|---|---|---|---|---|
| | | Leaves-AgNPs | Stem bark-AgNPs | Acarbose |
| 1 | 20 (µg/ml) | 48.83±0.824 | 58.74±0.832 | 70.27±0.808 |
| 2 | 40 (µg/ml) | 50.63±0.832 | 62.35±0.832 | 74.77±0.808 |
| 3 | 60 (µg/ml) | 55.14±0.823 | 64.15±0.824 | 80.18±0.832 |
| 4 | 80 (µg/ml) | 60.74±0.824 | 68.95±0.832 | 81.98±0.824 |
| 5 | 100 (µg/ml) | 64.64±0.824 | 72.85±0.824 | 82.88±0.824 |

**At the 0.05 level, the population means are significantly different**

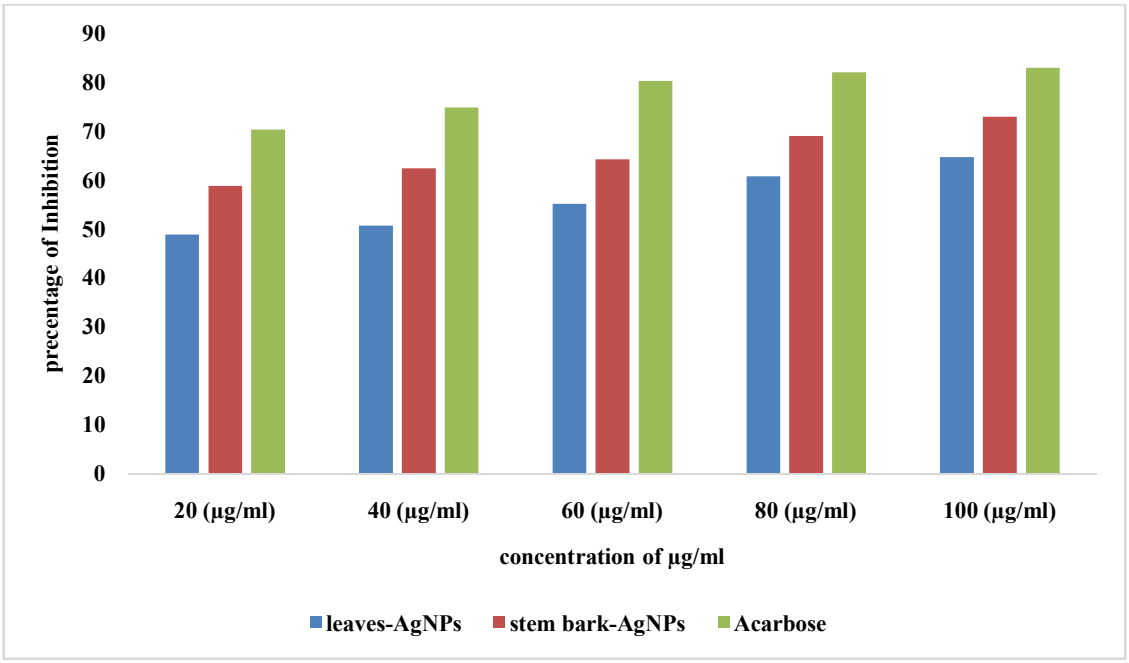



**Figure 9: α- glucosidase inhibitory activity of Acarbose vssilver nanoparticles synthesized using a *Pterocarpus indicus***

## CONCLUSION

From the present study, synthesis of silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* showed maximum inhibitory activity of antioxidant and antidiabetic activity under *in vitro* condition. Synthesized AgNPs from the *Pterocarpus indicus* are characterized using UV-Visible spectroscopy, zeta potential for particle size analysis and SEM analysis are spherical with sizes in the ranges from 37.9-126 nm. Alpha amylase inhibitory action decreases the digestion of carbohydrates and alpha glucosidase reduces glucose level in blood. As a result, we found that the synthesized leaves-AgNPs and stem bark-AgNPs have free radical scavenging activity and inhibitory activity against α-amylase and α-glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus.

## CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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# Phytochemical profiling and GC-MS analysis of *Pterocarpus indicus* willd. Using ethanolic stem bark and leaves extract

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**Abstract**
Nature has blessed us with enormous wealth of herbal plants which are widely distributed all over the world. *Pterocarpus indicus* belongs to the family Fabaceae is well known in Indian system for its medicinal uses. The present investigation was carried out to determine the possible bioactive components from the ethanolic stem bark and leaves extract of *Pterocarpus indicus* using preliminary phytochemical screening and GC-MS analysis, while the mass spectra of the compounds found in the extract was matched with the WILEY and National Institute of Standards and Technology (NIST Library). The results of the GC-MS analysis provide different peaks determining the presence of 20 phytocomponents in stem bark and 10 phytocomponents in leaves. The presence of these bioactive compounds in *Pterocarpus indicus* is an excellent source to treat various diseases and health complications in humans.

**Keywords:** *Pterocarpus indicus*, GC-MS analysis, ethanolic extract, WILEY, NIST library

## Introduction

*Pterocarpus indicus* is an important therapeutic and medicinal plant belonging to family Fabaceae [1]. The treasure of India is stored in the vast natural flora, which has been boon to mankind [2]. Various plants available in the nature are still unexplored for their medicinal potential. Among them, *Pterocarpus indicus* is one of the most valuable multipurpose forest trees. It is a large deciduous tree widely distributed in the central, western and southern regions of India, having a long history of numerous traditional and ethnobotanical applications in diverse cultures [3]. All parts of *Pterocarpus indicus* is used as a primitive medicine for domestic remedy against several human diseases. It has been used in Homeopathic, Ayurvedic and Unani systems of medicine [4-5]. The medicinal value of the plants lies in the bioactive phytochemical constituents that produce definite physiological effect on human body [6]. Phytomedicines derived from various parts of the plants are the important components with numerous applications in pharmaceutical industry [7]. The aim of this work was to investigate the bioactive constituents of *Pterocarpus indicus*, which may provide an insight about the use of traditional medicine.

## Materials and methods

### Collection of Plant Material

The *Pterocarpus indicus* bark and leaves were collected in the month of December from the Senthankudi Village, Pudukkottai, Tamil Nadu, India. The plant was identified and authenticated by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirappalli, Tamil Nadu for identifying the plants.

### Preparation of Plant Extract

*Pterocarpus indicus stem* bark and leaves *were separately* washed in running water, cut into small pieces and then shade dried for a week at 35-40$^0$C, after which it was grinded to a uniform powder of 40 mesh size. The extracts were prepared by soaking 100 g each of the dried powder plant materials in 1 L of ethanol using a soxhlet extractor continuously for 10 hrs. The extracts were filtered through whatman filter paper No.42 (125mm) to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labeled sterile bottles and kept at -40$^0$C. The filtrate obtained was used as sample solution for the further isolation [8-9].

### Preliminary Phytochemical Screening

Phytochemical analysis of ethanolic stem bark and leaves extract of *Pterocarpus indicus* was carried out as described by (*Harborne 1988*). By this analysis, presence of several phytochemicals like tannin, phlobatannin, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, leuco anthocyanin, anthocyanin, anthroquinone, coumarin, glycosides, phenol, xanthoproteins, alkaloids, emodine, carbohydrate and proteins were tested [10-11].

### Identification of Components

Gas chromatography–mass spectrometry (GC-MS) interpretation was conducted using the database of WILEY and NIST. Spectrum of the unknown component was compared with the spectrum of known components stored in the WILEY and NIST Library.

### GC-MS Analysis

GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) system was utilized. The system was equipped with an auto injector (AOC-20i), head space sampler (AOC-20s), a mass selective detector with an iron source (220 °C) and an interface (260 °C). Rtx-5 MS capillary column having 30 mm X 0.25 mm of length X diameter and 0.25 µm of film thickness was used for MS analyses. The mass range of 40-650 m/z with 1,000 ev of the threshold was purposed. The injector was set in the split injection mode having 250 °C of temperature. The ratio applied for split mode was 10.0. The starting temperature was adjusted to 80 °C (3 min), which afterwards increased to 280 °C with a ramp rate of 10 °C/min. Helium (>99.99 %) with 40.5 cm/s of linear velocity was employed as a carrier gas. The system was programmed with 16.3 ml/min of total flow rate and 1.21 ml/min of column flow. Components were recognized by their retention time (RT) and elucidation of mass spectra. The spectral fragmentation of unknown components was compared with the known and standard components provided by the databases of WILEY and NIST Library.

## Results

### Preliminary Phytochemical Screening

Qualitative analysis of ethanolic stem bark and leaves extract of *Pterocarpus indicus* showed positive response for tannin, phlobatannin, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, leuco anthocyanin, anthocyanin, anthroquinone, coumarin, glycosides, phenol, xanthoproteins, alkaloids, emodine and carbohydrates (Table:1). Quantitative analysis of important phyto chemicals in *Pterocarpus indicus* contains phyto chemicals in varying amounts in the bark. The phytochemicals with the highest quantity was tannin (0.053mg/g) followed by flavonoids (0.018mg/g), saponin (0.02mg/g), alkaloids (0.025mg/g), phenols (0.019mg/g), terpenoids (0.012mg/g) respectively and leaves also revealed flavonoids (0.036mg/g), tannin (0.083mg/g), saponin (0.051mg/g), alkaloids (0.057mg/g), phenols (0.010mg/g), terpenoids (0.028mg/g) respectively (Table: 2).

**Table 1:** Qualitative Analysis of Ethanolic Stem Bark and Leaves Extract of *Pterocarpus indicus*

| S. No | Phytochemical Constituents | Observation | Stem Bark | Leaves |
|---|---|---|---|---|
| 1 | Tannin | Brownish green | +++ | +++ |
| 2 | Phlobatannin | Red precipitate | +++ | + |
| 3 | Saponin | Foam | +++ | +++ |
| 4 | Flavonoids | Yellow colour | +++ | +++ |
| 5 | Steroids | Blue colour | ++ | +++ |
| 6 | Terpenoids | Reddish brown precipitate | +++ | +++ |
| 7 | Cardiac glycosides | Brown ring | +++ | +++ |
| 8 | Leuco anthocyanin | Red layer | + | + |
| 9 | Anthocyanin | Bluish violet | ++ | + |
| 10 | Anthroquinone | Red colour | +++ | + |
| 11 | Coumarin | Yellow colour | +++ | +++ |
| 12 | Glycosides | Green colour | ++ | +++ |
| 13 | Phenol | Blue black | +++ | +++ |
| 14 | Xanthoprotein | Reddish orange precipitate | ++ | + |
| 15 | Alkaloids | Yellow colour | +++ | +++ |
| 16 | Emodine | Red colour | +++ | ++ |
| 17 | Carbohydrate | Reddish violet ring | +++ | ++ |

(+ = Slightly Present, ++ = Moderately Present, +++ = Strongly Present)

**Table 2:** Quantitative Analysis of Ethanolic Stem Bark and Leaves Extract of *Pterocarpus indicus*

| S. No | Phytochemical Constituents | Bark (mg/g) | Leaves (mg/g) |
|---|---|---|---|
| 1. | Flavonoids | 0.018 | 0.036 |
| **2.** | Tannin | 0.053 | 0.083 |
| 3. | Saponins | 0.020 | 0.051 |
| 4. | Alkaloid | 0.025 | 0.057 |
| 5. | Phenol | 0.010 | 0.024 |
| 6. | Terpenoids | 0.012 | 0.028 |

### GC-MS Analysis

Gas Chromatography–Mass Spectrometry (GC-MS) is a valuable tool for reliable identification of bioactive compounds. In the present study, 20 bioactive compounds have been identified from the ethanolic extract of *Pterocarpus indicus* stem bark and 10 bioactive compounds have been identified in leaves by GC - MS analysis. The active principles with their Retention Time (RT), molecular formula, molecular weight and peak area in percentage are presented in Table: 3 & 4.

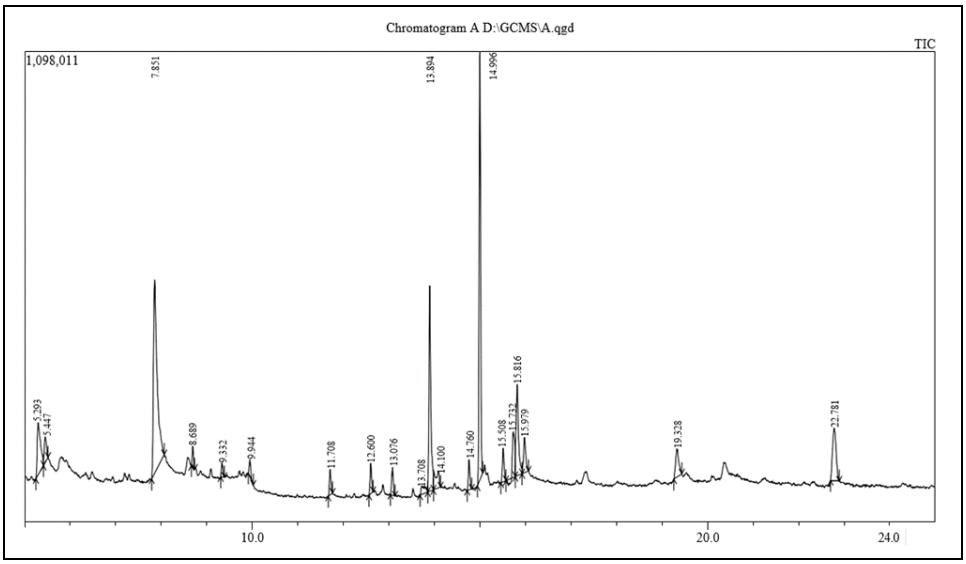


**Fig 1:** GC-MS chromatogram of the ethanolic extract of *Pterocarpus indicus* bark

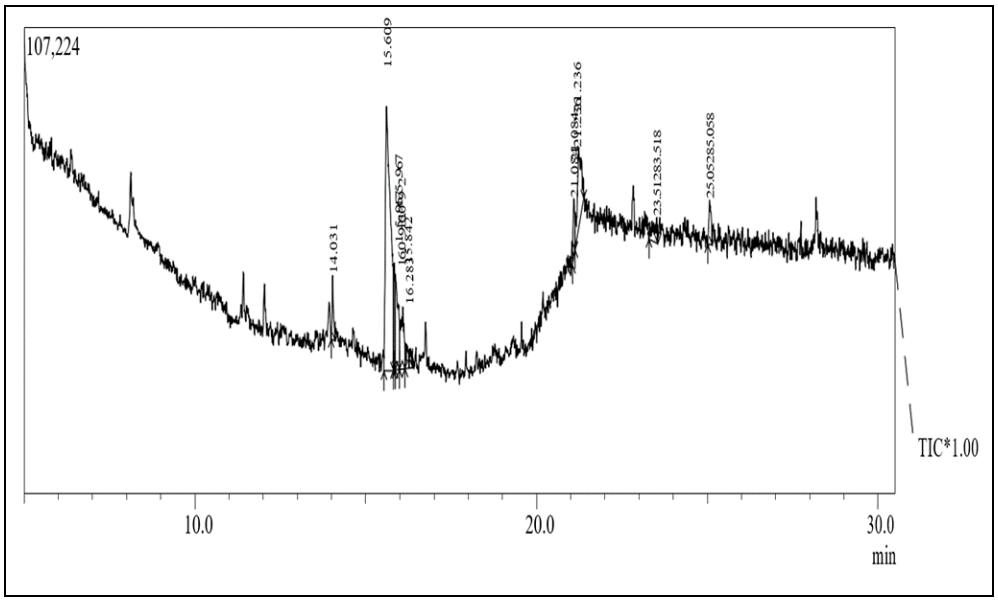


**Fig 2:** GC-MS chromatograms of the ethanolic extract of *Pterocarpus indicus* leaves

**Table 3:** Bioactive compounds detected in ethanolic bark extract of *Pterocarpus indicus*

| S. No | R. Time | Area% | Height% | Compound Name | Molecular Formula | Molecular Weight |
|---|---|---|---|---|---|---|
| 1 | 5.293 | 5.55 | 3.77 | 4-Hepten-3-one, 4-methyl- (CAS) 4-Methyl | $C_8H_{14}O$ | 126 |
| 2 | 5.447 | 1.83 | 1.85 | 1,2,3-Propanetriol, diacetate (CAS) Diacetin | $C_7H_{12}O_5$ | 176 |
| 3 | 7.851 | 23.55 | 14.02 | Cytidine (CAS) Cyd | $C_9H_{13}N_3O_5$ | 243 |
| 4 | 8.689 | 0.87 | 1.51 | Zingiberene | $C_{15}H_{24}$ | 204 |
| 5 | 9.332 | 0.69 | 1.02 | Hexadecanoic acid (CAS) Palmitic acid | $C_{16}H_{32}O_2$ | 256 |
| 6 | 9.944 | 0.85 | 1.25 | Benzoldicarbonsaeure | $C_{20}H_{26}O_4$ | 330 |
| 7 | 11.708 | 1.27 | 1.87 | Dodecanoic acid (CAS) Lauric acid | $C_{12}H_{24}O_2$ | 200 |
| 8 | 12.600 | 1.51 | 2.20 | Cyclopentane, heneicosyl- (CAS) Heneicosane | $C_{26}H_{52}$ | 364 |
| 9 | 13.076 | 1.48 | 2.02 | 2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol | $C_9H_{18}O$ | 142 |
| 10 | 13.708 | 1.21 | 0.51 | 2-(4-hydroxy-2-butenyl)-2-nitrocycloheptanon | $C_{11}H_{17}NO_4$ | 227 |
| 11 | 13.894 | 12.50 | 14.92 | Nonadecanoic Acid | $C_{19}H_{38}O_2$ | 298 |
| 12 | 14.100 | 2.28 | 0.94 | Phthalic acid, butyl ester, ester with butyl glyc | $C_{18}H_{24}O_6$ | 336 |
| 13 | 14.760 | 1.49 | 2.18 | Bi-1,3,5-cycloheptatrien-1-yl (CAS) | $C_{14}H_{12}$ | 180 |
| 14 | 14.996 | 21.33 | 31.11 | 5,8,11,14-Icosatetraynoic Acid | $C_{20}H_{24}O_2$ | 296 |
| 15 | 15.508 | 1.82 | 2.44 | dl-Citronellol | $C_{10}H_{20}O$ | 156 |
| 16 | 15.732 | 3.22 | 3.32 | 1, E-11, Z-13-Octadecatriene | $C_{18}H_{32}$ | 248 |
| 17 | 15.816 | 7.04 | 6.65 | 9,12,15-Octadecatrienoic acid, methyl ester | $C_{19}H_{32}O_2$ | 292 |
| 18 | 15.979 | 2.51 | 2.55 | 9-Octadecenoic acid (Z)- (CAS) Oleic acid | $C_{18}H_{34}O_2$ | 282 |
| 19 | 19.328 | 2.91 | 2.05 | Trans-2-Phenyl-1,3-Dioxolane-4-M | $C_{28}H_{40}O_4$ | 440 |
| 20 | 22.781 | 6.11 | 3.79 | 1,2-Benzenedicarboxylic acid, bis (2-ethylhexy | $C_{24}H_{38}O_4$ | 390 |
| | | 100.00 | 100.00 | | | |

**Table 4:** Bioactive compounds detected in ethanolic leaves extract of *Pterocarpus indicus*

| S. No | R. Time | Area% | Height% | Compound Name | Molecular Formula | Molecular Weight |
|---|---|---|---|---|---|---|
| 1 | 14.031 | 3.31 | 8.16 | Docosane | $C_{22}H_{46}$ | 310 |
| 2 | 15.609 | 48.92 | 33.61 | Phthalic acid, di-(1-hexen-5-yl) ester | $C_{20}H_{26}O_4$ | 330 |
| 3 | 15.842 | 5.36 | 13.35 | Propanoyl chloride | $C_3H_5ClO$ | 92 |
| 4 | 15.967 | 9.47 | 8.19 | Methane, Thiobis- | $C_2H_6S$ | 62 |
| 5 | 16.092 | 7.72 | 7.63 | 1,3-dioxolane, 2-(phenylmethyl)- | $C_{10}H_{12}O_2$ | 164 |
| 6 | 16.283 | 3.45 | 2.51 | n- Allyloxymethyl acrylamide | $C_7H_{11}N\ O_2$ | 141 |
| 7 | 21.084 | 3.59 | 7.53 | Silane, [1-(5-hexenyl)-2methylenecyclopropy | $C_{13}H_{24}S_i$ | 208 |
| 8 | 21.236 | 11.29 | 10.13 | 14-Heptadecenal | $C_{17}H_{32}O$ | 252 |
| 9 | 23.518 | 3.44 | 3.28 | 4,6-Decadiyn-3-ol, 3-isopropyl-9- (methoxyeth | $C_{17}H_{28}\ O_4$ | 296 |
| 10 | 25.058 | 3.44 | 5.62 | Silicate anion tetramer | $C_{24}H_{72}O_{12}\ S_{i12}$ | 888 |
| | | 100.00 | 100.00 | | | |

**Table 5:** Biological activity of phyto-components identified in the ethanolic stem bark and leaves extract of *Pterocarpus indicus*

| Phytoconstituents | Biological Activity |
|---|---|
| Hexadecanoic acid (CAS) Palmitic acid | Antioxidant, Hypocholesterolemic, Flavor, Antiandrogenic Pesticide, Nematicide, Lubricant. [12] |
| Benzoldicarbonsaeure, di-(hex-1-en- | Antimicrobial, Antifouling [12] |
| Phthalic acid, butyl ester, ester with butyl glyc | Antimicrobial, Antifouling [12] |
| 9,12,15-Octadecatrienoic acid, methyl ester | Anti- inflammatory, Insectifuge, Hepatoprotective, Cancer preventive, Antihistaminic. [12] |
| Zingiberene | Antimicrobial, Anti- inflammatory, Blood pressure-lowering, Cholesterol-lowering, Antiplatelet aggregation, Chemo preventive, Antioxidant, and Hypoglycemic properties. [13-14] |
| Dodecanoic acid (CAS) Lauric acid | Antibacterial |
| Nonadecanoic Acid | Nematicide, Lubricant, Antioxidant, Hypocholesterolemic, Cancer Preventive. [12] |
| Docosane | Antibacterial and Antioxidant [15] |
| 1,3-dioxolane, 2-(phenylmethyl)- | Antimicrobial [16] |
| 14-Heptadecenal | Antimicrobial [16] |
| 9-Octadecenoic acid (Z)- (CAS) Oleic acid | Antioxidant, Cell Proliferation and Prevents the Pro-Apoptotic Effect [17-18] |

## Discussion

The present study shows that the qualitative analysis reveals flavonoids, tannins, saponins, alkaloid, phenol, terpenoids, Cardiac glycosides and Coumarin are strongly present in both stem bark and leaves extract, hence quantitative test were performed for flavonoids, tannins, saponins, alkaloid, phenol and terpenoids. The leaves extract shows the increased concentration of tannin (0.083mg/g), alkaloid (0.057mg/g) and saponins (0.051mg/g) and the stem bark shows the increased concentration of tannin (0.053mg/g), alkaloid (0.025mg/g) and saponins (0.020mg/g) respectively. These secondary metabolites serve as defence mechanism against different infectious diseases. The phytoconstituents such as, phenols and flavonoids were reported for enhancing the scavenging of free radicals [19]. Natural antioxidant compounds are considered to be an endogenous defence mechanism as a protection against oxidative stress due to extreme level of environmental conditions [20]. GC-MS analysis of ethanolic stem bark and leaves extract of *Pterocarpus indicus* revealed the presence of some important bioactive compounds. 5, 8, 11, 14-Icosatetraynoic Acid, Nonadecanoic Acid and Cytidine are present in the increased concentration in stem bark. Phthalic acid and 14-Heptadecenal are present in the higher concentration in leaves extract, which shows antioxidant, anticancer, antimicrobial, hypoglycemic hypo cholester olemic and anti-inflammatory properties that has been already reported. According to the previous studies the main chemical constituent of *Pterocarpus indicus are* glucosidal tannin namely kinotannic acid. Several other chemical constituents like pterostilbene, (-)-epicatechin, pterosupin, marsupsin, etc. have been identified and isolated. It also shows promising results in treatment of cataract [21]. And hypertriglyceridaemia [22]. This plant also finds its use as cardiotonic [23-24], hypoglycaemic [25], antihyperinsulinaemic [26], antifungal [27], cox-2 inhibitor [28]. And hepatoprotective agent [29]. *Pterocarpus indicus* is widely used in 'Ayurveda' as 'Rasayana' for management of various metabolic disorders including hyperlipidemia [30-31]. Also used to improve eye sight [32]. In combination with other drugs the wood is used to treat snake bites and scorpion stings [33]. Its toxicity, wound healing potential also evaluated in animal studies [34] and also show antioxidant and antimicrobial activity [35].

## Conclusion

The present study revealed that the ethanolic stem bark and leaves extract of *Pterocarpus indicus* found to be phyto pharmaceutical important plant, which is traditionally used for the treatment of various ailments and its medicinal application can be easily noticed by the presence of different bioactive molecules. In addition, further research is needed to identify and purify the active compounds responsible for therapeutic activity.

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