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ORIGINAL ARTICLE

Comprehensive metabolite profiling and therapeutic potential of black gram (*Vigna mungo*) pods: conversion of biowaste to wealth approach

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Abstract
The risk faced by the drug-resistant pathogens, research, and development for viable alternative medicine is gaining traction. This study aims to utilize agricultural waste beneficially, by investigating the methanol, ethanol, acetone, ethyl acetate, petroleum ether, and hexane extracts of black gram pods by gas chromatography-mass spectrometry (GC–MS), and Fourier transform infrared (FT-IR) analysis to identify metabolites and functional groups and to evaluate its antibacterial and antibiofilm potential on various fish disease-causing drug resistant pathogens like *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Numerous compounds were identified as major peak area percentage by GC–MS analysis based on the polarity. Methanolic and ethanolic extracts of black gram pods showed higher phenolic and tannin content compared to other solvents, these results correlate with antioxidant potential. IC_{50} values of both 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) by the methanolic extracts possessed 933.807 and 976.285 µg/mL respectively. All the extracts possessed potential antibiofilm activity against *A. hydrophila*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* in a dose-dependent manner. This study clearly shows that phenolic content is the major source for the inhibition of bacterial cell adherence (biofilm) against pathogens. Extraction in highly polar solvents exhibited higher content of phenols and tannins as compared to non-polar solvents. Findings of the current study support black gram pods as an excellent alternative medicine against fish disease-causing pathogens. It is proved in this study that the biowaste black gram pods could be recycled for the welfare of humans as well as for the growth of the country's economy.

Keywords Black gram pods · Antioxidant · Antibacterial · GC–MS · Tannin and phenolic content

1 Introduction

Metabolites comprise aromatic rings with hydroxyl groups that are known as phenolic and flavonoid compounds [1]. Hydroxyl radicals of phytochemicals can directly exhibit antioxidant activity through the phenolic substances, which are excellent electron donors [2]. Both edible and non-edible portions of plants contain considerable amounts of phenolic compounds [3]. Plant products possess high phenolics, and thus fruits, herbs, vegetables, and other plant materials are increasingly being used in industrial phenolic extraction. Using important horticultural crops that are also human food resources for phenolic production or extraction poses significant legal and cultural concerns [4]. For emerging and developing countries, this would be a very expensive endeavor. Increased phenolic production might divert important fruits and vegetables from the food supply. Bioactive phenolic metabolites could also be synthesized or extracted from agro-industry wastes. Every year, large quantities of these materials such as seeds, peels, pods, and husks are created as wastes and are either inadequately collected or left to decompose on the ground [5–7]. These materials are gaining more attention as readily available and inexpensive renewable sources for the synthesis of value-added compounds [8]. Numerous investigations have shown that bioactive compounds may be recovered from food processing or plant

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harvesting by-products such as banana peels [9], cauliflower wastes [10], garlic husks [11], and broccoli wastes [12].

The disease outbreaks are the most common difficulty in the process of freshwater fish farming [13]. *Aeromonas hydrophila, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Klebsiella pneumoniae* are the most disease-causing pathogens in freshwater fish. Antibiotics are widely used to treat diseases in fish farming [14, 15]. Rasul and Majumdar. [16] reported that long-term usage and unsuitable dosages of antibiotics may have negative impacts on environmental consequences. To overcome these limitations, natural phytochemicals isolated from plant materials could be an excellent alternative to antibiotics [17, 18]. Plant-based metabolites evolved to act as ligands for a diverse set of molecular targets, resulting in a high level of molecular promiscuity [19]. This multi-target characteristic is critical for phenols with antibacterial potential and synergistic effects with conventional antibiotics [20]. Black gram (BG) (*Vigna mungo*) is an Indian-originated fast-growing warm-season legume belonging to the *Fabaceae* family and genus *Vigna*. India is the world's largest source of BG, accounting for 70% of global gross domestic production [21]. BG is mainly cultivated in Tamil Nadu (southern part of India) and it has diversified biological properties like nutritive, bulk enhancer, diuretic, and aphrodisiac [22]. As a consequence, a massive chunk of black gram pods (BGP) is disposed in landfills. Phenolic acids may be found in a wide range of plant-based meals, with the largest amounts found in seeds, fruit peels, husks, shells, pods, and vegetable leaves. They are often found in combined forms such as amides, esters, or glycosides that are rarely seen in free form [23]. These phenolic acids are non-flavonoid polyphenolic compounds that can be further classified as hydroxybenzoic acid and hydroxycinnamic acid derivatives [24, 25]. Most phenolic acids found in legumes are associated with cellulose, protein, lignin, or smaller biomolecules like sugar, quinic acid, and maleic acid via ester, ether, or acetyl bonds which are released through alkali, acid, and enzymatic hydrolysis [26]. Black gram seed husks and pods have greater levels of phytohormone C-glycosyl flavones such as vitexin and isovitexin, which have anticancer properties by protecting DNA and blood platelets from lipid peroxidation [27]. Approximately 2–3 kg of pods yielded 1 kg of black gram (from crop protection guide, Tamil Nadu Agricultural University, Tamil Nadu, India). Production-related factors like seed weight, number of seeds per pod, and seed indices have a big impact on seed yield/plant [28]. Since various solvents produce extracts with varying antioxidant activity, the ability of extracts to prevent lipid oxidation may vary [29]. To our knowledge, no previous research has been conducted to evaluate black gram pods (BGP) or their features. Therefore, the present study is conducted to investigate the chemical composition of the various extracts of black gram pods by using GC–MS and FT-IR for the identification of metabolites, and functional groups respectively and to analyze its total phenolics, total tannins, and in-vitro antioxidant, antibacterial and biofilm inhibitory potential.

2 Materials and methods

2.1 Collection of black gram pod wastes and preparation of extracts

Black gram pod (BGP) wastes were collected from Nemmeli village (10°55′04.0″N 79°37′16.0″E) in Tiruvarur district, Tamil Nadu, India. The black gram pods were harvested, and shade dried at 37 °C before being coarsely pulverized. The powder was sieved with 0.2-mm sieve plates and stored at − 20 °C for later use. The extracts were made from powdered BGP using a cold maceration procedure with six solvents (10:90 W/V): petroleum ether, methanol, ethyl acetate, ethanol, acetone, and hexane [30]. The solvents chosen for this study depend on the nature of the bioactive metabolites, type, and part of the plant [31]. Moreover, based on the polarity, these solvents used for this study are from least polar to higher polar. Non-polar solvents such as hexane (0.009) and petroleum ether (0.117) were used in non-polar compound extraction, whereas polar solvents such as methanol (0.762) and ethanol (0.654) were utilized in polar compound extraction. And the mid-polar solvents (partially polar/non-polar) like acetone (0.355) and ethyl acetate (0.228) were used to extrude mid-polar compounds [32]. The filtrate was collected via Whatman No. 1 filter paper prior to getting concentrated at around 40 °C in a rotary vacuum evaporator under lower pressure until agglomerates were obtained. To remove superfluous solvents, the extracts were dried and stored at 4 °C for future investigations.

2.2 Primary Phytochemical analysis

Steroids A total of 250 mL conc. H_2SO_4 was added slowly after 0.5 mL crude extract was combined with 2 mL chloroform. The layer formed by H_2SO_4 turned yellow coloured green fluorescence, whereas the upper layer turned red indicating the presence of steroids [33].

Terpenoids A total of 2 mL chloroform was added to 1 mL crude extract. Then 2 mL of conc. H_2SO_4 was cautiously added and gently shaken. The presence of a reddish-brown steroidal ring demonstrates the existence of terpenoids [33].

Reducing Sugar In a test tube, 2 mL of the extract solution was added to 5 mL equal volumes of Fehling's solutions I and II and heated for 2 min in a water bath. The prevalence

of reducing sugars was shown by the brick-red precipitate [34].

Alkaloids Various extracts were filtered after being mixed with 2 mL dilute Hydrochloric acid. And in 2 mL of filtrate, few drops of Hager's reagent were added. The presence of bright yellow precipitate indicated that the test was positive [35–37].

Flavonoids A total of 2–3 mL of extract filtrate was treated with a piece of magnesium ribbon along with 1 mL of conc. HCl. The presence of flavonoids was detected by the pink-red/crimson colouring of the solution [38].

Saponins In a test tube, 1 mL of crude extract was combined with 5 mL of distilled water and violently shaken for 30 s. The existence of saponins was determined by the production of stable foam formation [33].

Tannin The occurrence of tannins was determined by mixing 1 mL of the extract with few drops of freshly produced ferric chloride (5%) and observing the development of a bright blue or bright green colour [33].

Phenol Few drops of 5% lead acetate were added to 1 mL of crude extract, and the formation of yellow-coloured precipitate showed the existence of phenol [39].

Anthraquinones Five milligrams (5 mg) of the powdery extract was warmed in a water bath for 5 min with 10% HCl. After that, it was filtered and cooled down. To the filtrate, an equal volume of $CHCl_3$ was added followed by few drops of 10% NH_3, and then the mixture was gently heated. The appearance of pink colour confirmed the presence of anthraquinones [34].

2.3 Characterization of the BGP extracts

2.3.1 FT-IR analysis

The KBr pellet method was employed to investigate the functional groups associated with the BGP extracts using a Fourier transform infrared (FT-IR) spectrophotometer (Perkin Elmer, USA) in spectra of 4000–500 cm^1.

2.3.2 GC–MS analysis of BGP extracts

Gas chromatography–mass spectrometry examination of BGP extracts was performed using a Shimadzu (QP2020) coupled with a mass spectrometer. A 30 m long, 0.25 mm inner diameter, and 0.25 mm film thickness SH-Rxi-5Sil-MS capillary column covered with 100% polydimethylsiloxane was adopted. The oven temperature was fixed at 50 °C initially, and then steadily increased to 280 °C at a rate of 6 °Cmin^{-1}, with a final hold time of 2 min. The temperature of the injector was 250 °C. At a pressure of 68.1 kPa, helium was used as the gas phase, with a flow rate of 1.2 mL/min and a linear velocity of 39.7 cm/s. In total, 100 µL of extracts was dissolved in the appropriate solvents and filtered through a syringe filter (0.25 µm) to remove contaminants. The prepared sample was also put into GC with a 1:10 split ratio. At 70 eV, the mass spectrum was obtained via electron ionization. The ion source was applied at 200 °C constantly. To interpret the metabolites, the mass spectrum of each chemical detected in extract was interpreted and matched with reference spectra in the NIST 2005 MS collection [40]. The overall average area to total area ratio was used to calculate the relative % of each compound.

2.4 In vitro antioxidant activity

2.4.1 DPPH scavenging assay

The efficacy of BGP extracts to scavenge free radicals was investigated by exposing it to 2, 2-diphenyl-1-picrylhydrazyl (DPPH) using the Brand-Williams et al. [41] technique. In 96-well plate, 100 µL of BGP extracts were prepared at 200, 400, 600, 800, and 1000 µg/mL concentrations. The standard used was ascorbic acid (Vitamin-C). Each well acquired 100 µL of freshly prepared DPPH (1 mM) suspension. The solution was placed in the dark and incubated at 37 °C for 30 min. The shift in a hue of the solution from violet to yellow indicated that reactive oxygen species had been banished, and it was measured at 517 nm in Synergy HT Multimode Reader (Biotek, Winooski, USA). Following that, the percentage of scavenging capability of the BGP extracts was calculated using the following equation.

$$\%Scavenging = [Ac - As \div Ac] \times 100 \quad (1)$$

where Ac is the absorbance value of control, and *As* is the absorbance value of the sample (BGP extracts).

2.4.2 ABTS radical scavenging assay

The antioxidant capabilities of several BGP extracts were determined using the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical's cationic decolorization experiment [42]. In total, 7 mM ABTS was prepared in ethanol and blended with 88 mL potassium persulphate solution (140 mM) at dark room temperature (RT) condition, and the reaction mixture was incubated for 14 h. For each experiment, the ABTS solution was diluted in ethanol (1:89 v/v) to yield an absorbance of 734 nm. Then, 100 µL of different solvent extract concentrations (200, 400, 600, 800, and 1000 µg/mL) were mixed with 2.0 mL of $ABTS^+$ solution, accordingly. Then the reaction was incubated for

10 min before the absorption was assessed at 734 nm. Vitamin C was used as the reference. The formula was utilized to calculate the scavenging potential (1).

2.5 Determination of total phenol and total tannin content of the BGP extracts

The total phenolic content (TPC) of the BGP extract was measured using the modified Klompong and Benjakul technique [43]. A total of 100 µL of extract were mixed with 900 µL of deionized water. Then, 500 µL of Folin-phenol Ciocalteu's reagent was added to the mixture. Following a 5-min incubation period, 10 mL of 7.5% Na_2CO_3 solution was mixed properly and incubated for 30 min at RT. The absorbance was measured at 750 nm using a UV visible spectrophotometer Synergy HT Multimode Reader (Biotek, Winooski, USA). The total phenolic content of extracts was expressed as milligram gallic acid equivalent (GAE)/100 g using gallic acid as the standard.

The total tannin content (TTC) of the different BGP extracts was investigated using the Folin-Ciocalteu phenol reagent as examined by Amorim et al. [44]. In total, 100 µL of the extract was blended with 8.3 mL of double-distilled water in a test tube, and then 0.5 mL of Folin-Ciocalteu phenol reagent was added and kept at RT for 5 min. A total of 1 mL of 35% Na_2CO_3 solution was added to the test tube. The mixture was incubated at 25 ± 2 °C for 30 min after being well shaken. The absorbance was measured at 725 nm. As a blank, double distilled water was used. The total tannin content of the extracts was calculated using tannic acid as a reference and represented as milligram tannic acid equivalent (TAE)/100 g.

2.6 Assessment of antibacterial efficacy

2.6.1 Agar well diffusion method

The antibacterial effect of BGP extracts was tested using the agar well diffusion method [45] against *A. hydrophila, P. aeruginosa, S. aureus,* and *K. pneumoniae* fish microbial pathogens. These strains were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. A standard antibiotic, streptomycin (1 mg/mL), was used to calculate the inhibition zones. Inoculating a loopful of each tested bacteria in a 10 mL nutrient broth medium yielded the working culture, which was then cultured at 37 °C for 24 h. The agar plates were prepared with cultured pathogens. The BGP extracts were diluted in DMSO at doses of 250, 500, 750, and 1000 µg/mL in a sequential manner and loaded in wells formed in agar well plates and incubated overnight. The diameter of inhibition zones was used to determine antibacterial activity.

2.6.2 Determination of minimum inhibitory concentration

A sterile 96-well microtiter plate with resazurin as a cell growth indicator was used to determine the minimum inhibitory concentration (MIC) values for all BGP extracts [46]. By transferring 100 µL of nutrient broth in a 96-well microtiter plate, the experiment was carried out in a sterilized laminar airflow chamber. The first two rows were served as checkpoint. In the third row of the plate, a volume of 100 µL of various extracts (10 mg/mL) in 10% (v/v) DMSO was added, and serial dilutions were performed. Ten microliters of bacterial inoculum (10^6 CFU/mL) was added to each well and followed by 10 µL of resazurin solution (10 mg/mL). The microtiter plate was then gently wrapped in an aluminium wrapper to prevent bacterial culture dehydration, and the plates were incubated for 24 h at RT in an incubator. The wells were visually checked for color change and shifts from purple to pink or colorlessness were deemed positive. The MIC value was established by identifying the lowest concentration of extract at which color change occurred. To establish the average MIC value of the BGP extracts, all experiments were performed in triplicate.

2.6.3 Effects on growth of the various BGP extracts on microbial cultures

Using the modified procedure of Qayyum et al. [47], the growth of *A. hydrophila, P. aeruginosa, S. aureus*, and *K. pneumoniae* was investigated by comparing both MIC and sub MIC concentrations of BGP extract. Microbial cultures were loaded into tubes overnight to obtain final inoculum of 10^6 CFU mL^{-1}, followed by the addition of extracts depending on the MIC and sub-MIC values obtained and incubated at 37 °C. Growth was measured by a UV—visible spectrophotometer (Synergy HT Multimode Reader, Bioteck instrument, Winooski, VT, USA) by reading at 600 nm every 2 h for 24 h. The effect of the BGP extracts on bacterial growth was studied in triplicates with untreated microbial culture as a control.

2.6.4 Inhibition of biofilm formation and development

Various BGP extracts were tested for their ability to prevent cell attachments (Antibiofilm) using a slightly modified approach by Lewis Oscar et al. [48]. Solvent extracts concentrations vary from 200 to 1000 µg/mL were used for antibiofilm evaluation against *A. hydrophila, P. aeruginosa, S. aureus*, and *K. pneumoniae*. Each well of a 96-well microtiter plate was loaded with 100 µL

of BGP extracts and an equal volume of bacterial culture (10^6 CFU mL^{-1}) (total volume in each well was 200 µL). In blank wells, 200 µL of Mueller Hinton Broth (MHB) without bacterial inoculum was added. To allow the cells to adhere to the surface, the plates were cloaked loosely with parafilm. The plates were incubated at 37 °C for 8 h without agitation. Following incubation, the contents of each well were removed. To eliminate non-adherent cells of the microtiter plates, wells were washed thrice with sterilized distilled water before being oven-dried for 45 min at 60 °C. The adhered wells were stained with 200 µL of 1% crystal violet and incubated at RT for 15 min to confirm the biofilm formation. To eradicate the unabsorbed dye, the plates were washed thrice with sterilized distilled water. A total of 150 µL of 99% ethanol was added to the wells to remove the discoloration. Finally, 100 µL of the de-stained solution from the cultured plate was transferred to a new plate and its absorbance was read at 590 nm using a microplate reader (Synergy HT Multimode Reader, Biotek instrument, Winooski, VT, USA). Each experiment was performed in triplicates. The percentage of inhibition was calculated by using Eq. (1).

2.7 Statistical analyses

The data were analyzed with SPSS 16.0 software and expressed as the mean ± standard error of the mean (SEM) (SPSS, Chicago, IL, USA). A one-way analysis of variance (ANOVA) was used to compare the triplicate results. A $p < 0.05$ was used to evaluate the significant differences between the experimental and control groups followed by Duncan's Multiple Range (DMRT) as a post hoc test. Origin Pro 9.0 (OriginLab corp., Northampton, US) was used to create the graphs, which were then rendered using Graphpad prism (GraphPad Software, San Diego, CA, USA).

3 Results and discussion

3.1 Primary phytochemical screening

Phenols, tannins, saponins, alkaloids, flavonoids, reducing sugars, terpenoids, and steroids of various BGP extracts through primary phytochemical screening were shown in Table 1. All of the extracts contain phenols and tannins, whereas methanol, ethanol, and acetone extracts contain flavonoids. Methanol, ethanol, and hexane extracts include alkaloids, whereas terpenoids are present in all solvent extracts except hexane. Metabolites have a wide range of functions, including structural application, signal transduction control, communication processes, and the formation of photonic structures via complex molecular identity [49]. Antioxidant, anti-diabetic, anti-inflammatory, neuroprotective, anti-cancer, and gut microbiota–modifying properties were demonstrated by phenolic compounds of the plants [50]. Alkaloids isolated from various plants and seafood solid wastes possess antimicrobial activity and their various approaches confirmed the drug-targeted discovery [51]. Flavonoids can alter the key cellular enzymatic functions due to their anti-inflammatory, anti-oxidative, and anti-carcinogenic properties [52]. Tannins are used to heal wounds and may have antimicrobial, antiseptic, anti-inflammatory, and other cardio-protective properties [53]. Because of their possible antioxidant effects, flavonoids are utilized to treat cancer [54]. These bioactive compounds are found in most extracts naturally and have been demonstrated to have multiple bioactivities such as antioxidants, antibacterial and anti-inflammatory against human diseases [55, 56]. Secondary metabolites are used by plants to defend themselves against diseases. As a result, they have the ability to prevent the proliferation of particular microbes partially or totally. This type of activity is likely to extend to animal and human diseases as well [57].

Table 1 Primary phytochemical screening of various extracts of Black gram pods

S. No	Name of the phytochemicals	Methanol	Ethanol	Hexane	Acetone	Petroleum ether	Ethyl acetate
01	Steroids	-	-	-	+	-	-
02	Terpenoids	+	+	-	+	+	+
03	Reducing Sugar	+	+	-	+	-	+
04	Alkaloids	+	+	+	-	-	-
05	Flavonoids	+	+	-	+	-	-
06	Saponins	+	+	+	+	+	+
07	Phenolics	+	+	+	+	+	+
08	Tannins	+	+	+	+	+	+
09	Anthraquinone	-	-	-	-	-	-

(+ present,—absent)

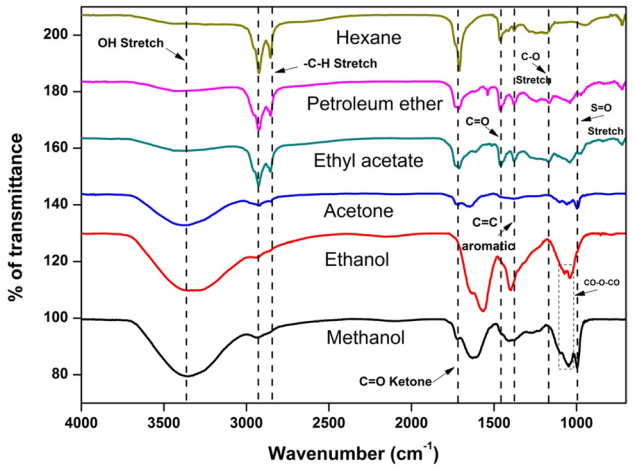


Fig. 1 FT-IR spectrum of black gram pod extracts

Table 2 Functional group of BGP extracts spectral peaks obtained through FT-IR analysis

S. No	Wavelength (cm^{-1})	Peak assignment	Functional group
1	3391.11	OH	Alcohols and phenols
2	2886	C–H	Alkenes
3	1603.15	C = O	Ketones
4	1482.57	N–H	Amide and amines
5	1398	C = C	Aromatic amines
6	1046	CO–O–CO (–C–H bend)	Anhydrides Alkane
7	1040	CO–O–CO (C–N)	Aromatic amines
8	1034.43	C-N	Aliphatic amines
9	1037.06	S = O	Sulfoxide
10	921.75	O–H	Carboxylic acids
11	765.51	C–H	Fatty acids and proteins
12	652.11	C–Br	Alkyl halides

3.2 FT-IR analysis

FT-IR spectroscopy has been used to detect the probable functional groups present in the various BGP extracts. Figure 1 shows the IR spectrum of extracts acquired in the range from 4000 to 500 cm^{-1}. Methanol, ethanol, and acetone extracts had a strong peak with high-intensity bands due to the polarity. The peak assignment and its functional groups were given in Table 2. The presence of the various spectrums indicated phytochemical metabolites like phenols, alkaloids, tannins, saponins, and flavonoids. The functional groups reported vibrational modes could be fragmentation linked to the basis of metabolites composition [58]. These findings were linked to the extracts' antioxidant and antibacterial capabilities owing to the existence of these functional groups [59]. It is possible to detect minute changes in primary and secondary metabolites, determine the structural modifications of some physiologically active molecules, and pinpoint those functional groups responsible for plant medicinal properties [60, 61]. FTIR spectroscopy has been demonstrated to be a proficient and sensitive tool for detecting biomolecule composition [62]. The obtained spectrum serves as metabolites identity, highlighting the many biochemical linkages and functional groups present in extracts as discrete peaks [63]. From the obtained results, the identification of the functional groups of the extract is based on the polarity of the solvent. Both the methanol and ethanol (highly polar) possessed similar spectra, and non-polar hexane and petroleum ether (non-polar) showed matching spectra [64] confirming that based on the polarity, functional groups were identified. Ketones were present in all the solvents whereas, the alcoholic group and aromatic amines were identified in solvents like methanol, ethanol, acetone, and ethyl acetate [65]. Alkenes were present in solvent extracts like ethyl acetate, petroleum ether, and hexane [66, 67].

3.3 GC–MS analysis of BGP extracts

GC–MS chromatogram detected the existence of metabolites in various BGP extracts were shown in Fig. 2. In this study, around 120 bioactive compounds were identified including hexane (20), acetone (30), petroleum ether (20), methanol (20), ethyl acetate (10), and ethanol (50). Major peak area (%) obtained compounds in hexane extracts were diacetone alcohol (66.44%) used as a pheromone compound in the cosmetics production [68], tetratetracontane (14.45%), a potential antibacterial [69] and antifungal agent [70]; acetone extracts were azulene (23.42%) acts as the anti-inflammatory agent [71]; anti-cancer activity [72]; anti-microbial property [73], 3,7,11,15-tetramethyl-2-hexadecen-1-OL (8.65%) possess anti-bacterial activity [74], methyl palmitate (4.29%) shows antimicrobial agent [75], -5-nonadecene (4.48%) exhibits anti-bacterial activity [76]; petroleum ether extract was 1-isopropoxy-2-propanol (14.44%) no biological reports available, methyl propyl ether (6.95%) no biological reports available, 3,7,11,15-tetramethyl-2-hexadecen-1-OL (4.74%) possess anti-bacterial activity [74]; tetracontane (14.52%) exhibits anti-fungal, anti-inflammatory property [77]; methanol extracts were naphthalene (38.25%) possess carcinogenic activity [78]; 1-hexadecene (8.29%) and cholesterol (14.76%) involve in homeostasis [79]; cholest-5-en-3-ol (3beta)- and propanoate (2.75%) no biological reports available, and methyl palmitate (1.35%) shows antimicrobial agent [75]; ethyl acetate extracts were tetratetracontane (68.40%), a potential antibacterial [69] and antifungal agent [70]; 1-hexadecene (3.27%); ethanol extracts were naphthalene (24.22%) possess carcinogenic activity

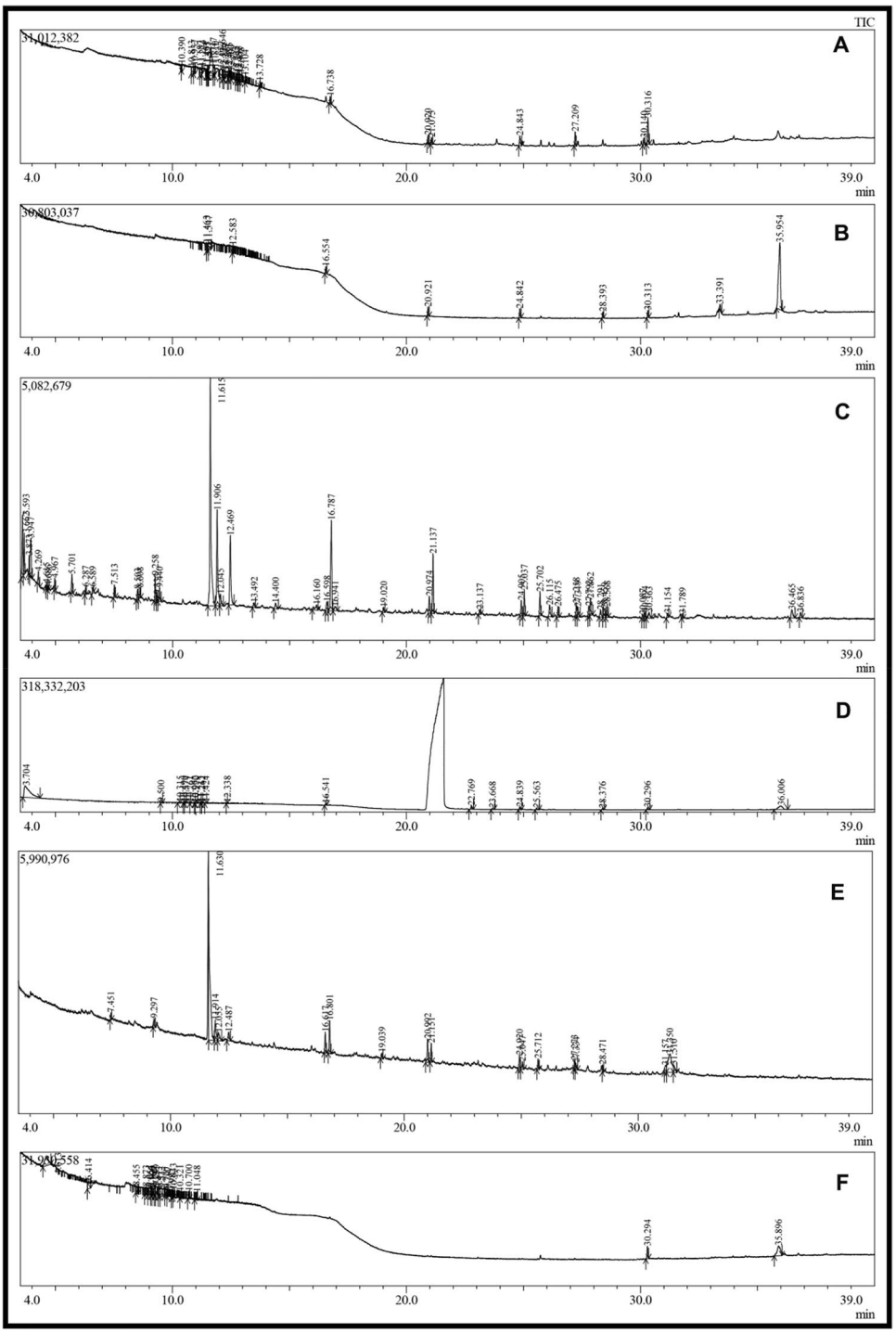


Fig. 2 GC–MS chromatogram of the various black gram pod extracts. **A** acetone, **B** ethyl acetate, **C** ethanol, **D** hexane, **E** methanol, **F** petroleum ether

[78]; 5-hydroxymethylfurfural (6.91%) exhibits antibacterial [80] and antifungal activity [81]; heptadecane (4.47%) shows antibacterial and antioxidant activity [82]; hexadecane (8.80%) possess antibacterial activity [83], respectively. These compounds are from many chemical classes, and most of them have been reported to have significant biological activity. These bioactive compounds have various functions like anti-basal activity, anti-microbial, antioxidants, anti-fungal, anti-inflammatory, and larvicidal. Multiple agents like an anti-microtubule agent, antibiofilm agent, anti-virulence agent, anti-Alzheimer's agent, anti-proliferative agent, and essential body metabolites (Tables 3, 4, 5, 6, 7, and 8). This discrepancy in bioactive compounds could be due to the compounds' volatility or the extraction solvent [84]. The GC–MS results from the various BGP extracts revealed that they all contained essentially the same

Table 3 GC–MS analysis of BGP hexane extract. (- Not available) *(Boldface compounds have higher peak area %)*

S. No	Name of the compound	Molecular formula	Molecular weight	Retention time	Peak area (%)	Biological applications
1	Diacetone alcohol	$C_6H_{12}O_2$	116.16	3.704	**66.44**	Pheromone compound [87]
2	3,7-Dimethyloctan-1-ol	$C_{10}H_{22}O$	158.28	9.500	1.30	Odorant binding protein [88]
3	3-Decylsulfinyltetrahydrothiophene-4-ol 1,1-dioxide	$C_{14}H_{28}O_4S_2$	324.5	10.315	0.90	Drug/therapeutic agent [89]
4	Butyronitrile	C_4H_7N	69.11	10.490	0.58	Antioxidant activity [90]; antimicrobial activity [91]
5	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	10.574	0.57	Synthetic used for drug optimization [92]
6	Butyronitrile	C_4H_7N	69.11	10.761	0.24	Anti-oxidant activity [90]; antimicrobial activity [91]
7	Butyronitrile	C_4H_7N	69.11	10.952	0.26	Anti-oxidant activity [90]; antimicrobial activity [91]
8	Butyronitrile	C_4H_7N	69.11	10.990	1.04	Anti-oxidant activity [90]; antimicrobial activity [91]
9	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	11.213	0.39	Synthetic used for drug optimization [92]
10	Diallyl oxalate	$C_8H_{10}O_4$	170.16	11.262	0.57	Anti-oxidant, anti-obesity and hepatoprotective property [93]
11	Butyronitrile	C_4H_7N	69.11	11.424	1.09	Anti-oxidant activity [90]; antimicrobial activity [91]
12	Butyronitrile	C_4H_7N	69.11	12.338	0.40	Anti-oxidant activity [90]; antimicrobial activity [91]
13	9-Octadecene	$C_{18}H_{36}$	252.5	16.541	1.13	Anti-dermatophytic activity [94]
14	1-O-Ethyl 2-O-propan-2-yl benzene-1,2-dicarboxylate	$C_{13}H_{16}O_4$	236.26	22.769	2.68	-
15	Phthalic acid, ethyl pentyl ester	$C_{15}H_{20}O_4$	264.32	23.668	1.09	Anti-microbial activity [95]
16	1-Heptadecene	$C_{17}H_{34}$	238.5	24.839	2.14	Repellant [96]
17	Phthalic acid, ethyl pentyl ester	$C_{15}H_{20}O_4$	264.32	25.563	1.77	Anti-microbial activity [95]
18	1-Nonadecene	$C_{19}H_{38}$	266.5	28.376	1.16	Anti-oxidant [97]; Anti-bacterial [98]
19	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296.5	30.296	1.81	Anti-inflammatory, anti-oxidant [99]; anti-microbial [100]; larvicidal activity [101]
20	Tetratetracontane	$C_{44}H_{90}$	619.2	36.006	**14.45**	Anti-babesial, anti-oxidant [102] and anti-inflammatory activity [103]

compound, which is attributable to the solvents' polarity [85, 86].

3.4 In vitro antioxidant potential

3.4.1 DPPH-scavenging assay

The antioxidant potential of several BGP extracts was revealed in this investigation. The ability of the different BGP extracts to effectively eliminate free radicals was identified based on the determination of the IC_{50} value (Table 9, Fig. 3a). Obtained IC_{50} value of ascorbic acid (94.65 µg/mL) followed by methanol (933.80 µg/mL), ethanol (1163.43 µg/mL), ethyl acetate (1145.65 µg/mL), acetone (1231.39 µg/mL), hexane (1330.32 µg/mL), and petroleum ether (1634.17 µg/mL). These findings are in accordance with the interaction between phenolic components and antioxidant ability of extracts. By regulating the generation of free radicals, the antioxidant defense system is important for managing a variety of chronic diseases [168]. The phenolic compounds are more soluble in polar solvents; hence they have a higher amount of phenol during extraction [169, 170]. The antioxidant concentration and efficacy are proportional to the amount of color change [171]. The phenolic concentration of the methanolic extract is greater, which can contribute hydrogen to a free radical to scavenge. In recent years, extensive research on the pharmacological actions of plant metabolites has

Table 4 GC–MS analysis of BGP Acetone extract. (- Not available) *(Boldface compounds have higher peak area %)*

S. No	Name of the compound	Molecular Formula	Molecular Weight	Retention time	Peak area (%)	Biological Applications
1	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	10.390	1.28	Synthetic used for drug optimization [92]
2	2-(4-Acetamidosulfanylphenyl)-2-[[(2S)-2-aminopropanoyl] amino]acetic acid	$C_{13}H_{17}N_3O_4S$	311.36	10.813	4.31	-
3	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	10.937	3.16	Synthetic used for drug optimization [92]
4	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	11.187	3.53	Synthetic used for drug optimization [92]
5	Butylamine	$C_4H_{11}N$	73.14	11.274	1.97	Anti-hypersensitive activity & Capping agent for nano-particles synthesis [104]
6	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	11.457	1.49	Synthetic used for drug optimization [92]
7	Propiolic acid	$C_3H_2O_2$	70.05	11.495	1.27	Anti-hypertensive activity [105]
8	3-Decylsulfinyltetrahydrothiophene-4-ol 1,1-dioxide	$C_{14}H_{28}O_4S_2$	324.5	11.527	125	Drug/therapeutic agent [83]
9	Azulene	$C_{10}H_8$	128.17	11.646	**23.42**	Cosmetic agent [106]
10	Azulene	$C_{10}H_8$	128.17	11.747	4.20	Cosmetic agent [106]
11	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	11.810	0.20	Synthetic used for drug optimization [92]
12	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	12.077	6.53	Synthetic used for drug optimization [92]
13	3-Decylsulfinyltetrahydrothiophene-4-ol 1,1-dioxide	$C_{14}H_{28}O_4S_2$	324.5	12.176	2.45	Drug/therapeutic agent [89]
14	3-Decylsulfinyltetrahydrothiophene-4-ol 1,1-dioxide	$C_{14}H_{28}O_4S_2$	324.5	12.247	3.26	Drug/therapeutic agent [89]
15	Butyronitrile	C_4H_7N	69.11	12.387	1.67	Anti-oxidant activity [90]; anti-microbial activity [91]
16	Bromocyclopropane	C_3H_5Br	120.98	12.425	2.96	-
17	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	12.500	0.26	Synthetic used for drug optimization [92]
18	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	12.725	2.12	Synthetic used for drug optimization [92]
19	3-Decylsulfinyltetrahydrothiophene-4-ol 1,1-dioxide	$C_{14}H_{28}O_4S_2$	324.5	12.797	2.01	Drug/therapeutic agent [89]
20	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	12.835	2.12	Synthetic used for drug optimization [89]
21	Butyronitrile	C_4H_7N	69.11	12.908	3.04	Anti-oxidant activity [90]; anti-microbial activity [91]
22	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	13.104	0.96	Synthetic used for drug optimization [92]
23	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	13.728	0.42	Synthetic used for drug optimization [92]
24	Tetradecane	$C_{14}H_{30}$	198.39	16.738	2.20	Dermal irritant [107]
25	1-Pentadecene	$C_{15}H_{30}$	210.40	20.920	2.19	Pheromone compound also used insect repellent [108]
26	Hexadecane	$C_{16}H_{34}$	226.44	21.075	1.91	Induces hyper Keratinization in tested rodents [109]
27	Z-5-Nonadecene	$C_{19}H_{38}$	266.5	24.843	4.48	Anti-oxidant activity [110];
28	Methyl palmitate	$C_{17}H_{34}O_2$	270.5	27.209	4.29	Anti-radical activity [111]; Anti-cancer activity [112]
29	8,11,14-Docosatrienoic acid, methyl ester	$C_{23}H_{40}O_2$	348.6	30.140	2.40	-

Table 4 (continued)

S. No	Name of the compound	Molecular Formula	Molecular Weight	Retention time	Peak area (%)	Biological Applications
30	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296.5	30.316	**8.65**	Anti-inflammatory, anti-oxidant [99]; anti-microbial [100]; larvicidal activity [101]

Table 5 GC–MS analysis of BGP Petroleum ether extract. (- Not available) *(Boldface compounds have higher peak area %)*

S. No	Name of the compound	Molecular formula	Molecular weight	Retention time	Peak area (%)	Biological applications
1	1-Isopropoxy-2-propanol	$C_6H_{14}O_2$	118.17	4.613	**14.44**	Anti-microtubule activity [113]
2	Methyl propyl ether	$C_4H_{10}O$	74.12	6.414	**6.95**	Anti-oxidant [114]; anti-proliferative activity [114]
3	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	8.455	3.93	Synthetic used for drug optimization [92]
4	Butyronitrile	C_4H_7N	69.11	8.873	3.79	Anti-oxidant activity [90]; anti-microbial activity [91]
5	4-Methyl-1,3-thiazol-2(3H)-one	C_4H_5NOS	115.16	9.054	6.10	-
6	Butyronitrile	C_4H_7N	69.11	9.100	2.63	Anti-oxidant activity [90]; anti-microbial activity [91]
7	2,5-Dimethylpyrazine	$C_6H_8N_2$	108.14	9.160	3.28	-
8	Butyronitrile	C_4H_7N	69.11	9.247	3.25	Anti-oxidant activity [90]; anti-microbial activity [91]
9	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	9.299	6.92	Synthetic used for drug optimization [92]
10	3-Decylsulfinyltetrahydrothiophene-4-ol 1,1-dioxide	$C_{14}H_{28}O_4S_2$	324.5	9.413	4.25	Drug/therapeutic agent [89]
11	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	9.533	10.84	Synthetic used for drug optimization [92]
12	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	9.720	3.36	Synthetic used for drug optimization [92]
13	3-Decylsulfinyltetrahydrothiophene-4-ol 1,1-dioxide	$C_{14}H_{28}O_4S_2$	324.5	9.797	1.70	Drug/therapeutic agent [89]
14	Butyronitrile	C_4H_7N	69.11	9.982	1.61	Anti-oxidant activity [90]; anti-microbial activity [91]
15	2,2-Difluorocycloheptan-1-one	$C_7H_{10}F_2O$	148.15	10.033	1.68	Synthetic used for drug optimization [92]
16	Butyronitrile	C_4H_7N	69.11	10.321	1.03	Anti-oxidant activity [90]; anti-microbial activity [91]
17	Diallyl oxalate	$C_8H_{10}O_4$	170.16	10.700	2.58	Anti-oxidant, anti-obesity and hepatoprotective property [93]
18	1,1-Difluoro-2-(trans-1-propenyl)cyclopropane	$C_6H_8F_2$	118.12	11.048	2.41	-
19	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296.5	30.294	**4.74**	Anti-inflammatory, anti-oxidant [99]; anti-microbial [100]; larvicidal activity [101]
20	Tetracontane	$C_{40}H_{82}$	563.1	35.896	**14.52**	Anti-oxidant and anti-microbial property [115]

linked the presence of antioxidants such as flavonoids, isoflavones, flavones, anthocyanin, catechin, and isocatechin to the main protective impact of secondary metabolites [172, 173]. Our data found a strong association between phenolic chemicals and antioxidant capability. Multiple hydroxyl functional groups found in phenolics, and flavonoids are thought to be responsible for their biological and antioxidant properties [174–176]. Phenolic substances also boost antioxidant enzyme activity, which has an indirect effect on the quantity of damaging oxygen radicals

Table 6 GC–MS analysis of BGP Methanol extract. (- Not available) *(Boldface compounds have higher peak area %)*

S. No	Name of the compound	Molecular formula	Molecular weight	Retention time	Peak area (%)	Biological applications
1	2-Ethylhexanol	$C_8H_{18}O$	130.23	7.451	1.10	-
2	Undecane	$C_{11}H_{24}$	156.31	9.297	1.76	Anti-allergic and anti-inflammatory agent [116]
3	Naphthalene	$C_{10}H_8$	128.17	11.630	**38.25**	Anti-alzheimer's agent [117]
4	Dodecane	$C_{12}H_{26}$	170.33	11.914	5.44	Skin irritant [118]
5	Decanal	$C_{10}H_{20}O$	156.26	12.055	2.81	Electroencephalographic activity [119]; anti-microbial agent [120]
6	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	12.487	2.54	Anti-biofilm, Anti-virulence activity [121]
7	1-Hexadecene	$C_{16}H_{32}$	224.42	16.617	3.77	Anti-microbial, anti-oxidant property [122]
8	Hexadecane	$C_{16}H_{34}$	226.44	16.801	6.03	Induces hyper Keratinization in tested rodents [109]
9	Pentadecane	$C_{15}H_{32}$	212.41	19.039	1.13	Anti-fungal activity [123]; hypertensive activity [124]
10	1-Hexadecene	$C_{16}H_{32}$	224.42	20.992	4.52	Anti-microbial, anti-oxidant property [122]
11	Hexadecane	$C_{16}H_{34}$	226.44	21.151	3.06	Induces hyper Keratinization in tested rodents [109]
12	1-Octadecene	$C_{18}H_{36}$	252.5	24.920	2.52	Anti-fouling property [125]
13	2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	240.5	25.047	1.63	-
14	Neophytadiene	$C_{20}H_{38}$	278.5	25.712	1.68	Anti-microbial property [126]
15	Methyl palmitate	$C_{17}H_{34}O_2$	270.5	27.273	1.35	Anti-radical activity [111]; Anti-cancer activity [127]
16	1,1'-Sulfonylbis(3,5-dibromo-4-(2,3-dibromopropoxy) benzene)	$C_{18}H_{14}Br_8O_4S$	965.6	27.334	1.67	-
17	1-Docosene	$C_{22}H_{44}$	308.6	28.471	1.01	Anti-cancer activity [128]; anti-oxidant activity [129]
18	(4As,7aS)-3-Methylhexahydrocyclopenta[e][1,3]oxazin-2(3H)-one	$C_8H_{13}NO_2$	155.19	31.157	**2.21**	-
19	Cholesterol	$C_{27}H_{46}O$	386.7	31.350	**14.76**	Essential body metabolite for humans, animals, plants, and other organism [130]
20	Cholest-5-en-3-ol (3beta)-, propanoate	$C_{30}H_{50}O_2$	442.7	31.510	**2.75**	Essential body metabolite for humans, animals, plants, and other organism [130]

in living cells. Radical processes, such as DNA damage and superoxide anion generation, can also serve as a pro-oxidant in large quantities [177, 178]. The methanolic extract of the BGP has more potent antioxidant activity than the other extracts, which is proportional to phenolic concentration among the extracts [179–182]. The major metabolites of BGP extract acts as the antioxidant agents are 1-Hexadecene [183]; Tetracontane [184, 185]; 1-Nonadecene [186]. According to Urbaniak et al. [187], the extracts' proton-donating capacity stabilizes free radicals in the presence of numerous hydroxyl groups, resulting in improved DPPH scavenging action.

3.4.2 ABTS radical scavenging assay

ROS inhibition was probably an adaptable mechanism to low levels of mitochondrial dysfunction. Such an adaptive response could be affected by a quick increase in antioxidant defense leads to a reduction in ROS [188]. Several BGP extracts were investigated for ABTS radicals scavenging activity (Fig. 3b). The IC_{50} value was used to determine the scavenging ability of the different BGP extracts (Table 9). In the ABTS experiment, the plant extracts can decrease cations. According to Michalak [189], the potential of BGP phytochemical constituents to scavenge free radicals and

Table 7 GC–MS analysis of BGP ethyl acetate extract. (- Not available) *(Boldface compounds have higher peak area %)*

S. No	Name of the compound	Molecular formula	Molecular weight	Retention time	Peak area (%)	Biological Applications
1	Cyanoacetic acid	$C_3H_3NO_2$	85.06	11.463	3.71	Anti-tumour activity [131]
2	Butyronitrile	C_4H_7N	69.11	11.547	**7.78**	Anti-oxidant activity [90]; anti-microbial activity [91]
3	Butyronitrile	C_4H_7N	69.11	12.583	2.19	Anti-oxidant activity [90]; anti-microbial activity [91]
4	1-Pentadecene	$C_{15}H_{30}$	210.40	16.554	2.62	Pheromone compound also used as insect repellent [108]
5	1-Hexadecene	$C_{16}H_{32}$	224.42	20.921	3.27	Anti-microbial, anti-oxidant property [132]
6	1-Heptadecene	$C_{17}H_{34}$	238.5	24.842	3.87	Repellant [96]
7	1-Nonadecene	$C_{19}H_{38}$	266.5	28.393	2.42	Anti-oxidant [97]; Anti-bacterial [98]
8	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296.5	30.313	3.05	Anti-inflammatory, anti-oxidant [99]; anti-microbial [100]; larvicidal activity [101]
9	Nonacosanal	$C_{29}H_{58}O$	422.8	33.391	2.68	Anti-cancer activity [133]
10	Tetratetracontane	$C_{44}H_{90}$	619.2	35.954	**68.40**	Anti-babesial, anti-oxidant [102]; anti-inflammatory activity [103]

prevent oxidation processes may be linked to their chemical composition, which includes hydroxyl groups and unsaturated bonds. Ascorbic acid (205.65 µg/mL) possesses least IC_{50} value than methanol (976.28 µg/mL), ethanol (1057.57 µg/mL), ethyl acetate (1292.32 µg/mL), acetone (1338.74 µg/mL), hexane (1592.8 µg/mL), and petroleum ether (1413.70 µg/mL). These findings correlate with the phenol content of the methanolic extract, which is presently higher than other extracts [190]. Phenolics include one or more aromatic rings with many hydroxyl groups, so they can absorb free radicals [191, 192]. Total phenolic content was attributed to the occurrence of phenoxyl radicals with resonance stabilisation in the majority of extracts and antioxidant activity [193–195]. The BGP extracts possess antioxidant capability due to the presence of the metabolites like tetratetracontane [196, 197] and methyl linolelaidate [198].

3.5 Determination of total phenol and total tannin content of the BGP extracts

3.5.1 Total phenol content

The polarity of the solvents may alter the extraction of the phenol component. According to our results data, higher phenolic content was obtained in methanol (39.37 mg/GAE g), followed by ethanol (30 mg/GAE g), acetone (12.25 mg/GAE g), ethyl acetate (4.83 mg/GAE g), petroleum ether (3.43 mg/GAE g), and hexane (3.37 mg/GAE g) given in Fig. 4b. The total phenol content found in different extracts of BGP was reported in Table 10 and it was determined by the regression equation ($y = 0.0037x + 0.121$, $r^2 = 0.965$) of the Gallic acid equivalent (GAE) given in Fig. 4a by our investigation. Phenolic acids, flavonoids, and anthocyanins are the main components of total phenol content (TPC). Furthermore, differences in total phenol (TP) extraction operations, which included organic solvent, extraction time, temperatures, and auxiliary procedures (e.g., ultrasounds) in certain cases, or prior treatments such as irradiation or lyophilization, could be partially to censure for these disparities [199]. Plant extracts contain phenolic compounds, which are natural antioxidants that scavenge free radicals and reduce oxidative stress [200, 201]. Due to genetic and environmental conditions, the plant's phenolic content might fluctuate [202, 203]. The variation in phenol contents depends on the polarity of the extraction solvent [204]. The variances can be attributed to differences in solvent polarity, which selectively recover distinct hydrophobic or hydrophilic phenolic metabolites in the sample, emphasizing the necessity of researching and determining the best solvent for each sample [205] even though, no previous research has been performed on the phenolic profile of the various extracts of black gram pod wastes. While comparing with fresh tissues, the extracts have the greatest average of total phenolics, carotenoids, and total ascorbic acid. The oxidation of phenols by phenol oxidases and the polymerization of free phenols cause the phenol content of the extracts to decrease [206]. As they are more inclined to the synthesis of phenolic compounds, the outer parts of plants and fruits have a significant quantity of phenols [207]. The selection of solvents is the major criterion for efficient extraction [208]. Compounds identified in both methanol and ethanol solvents possessed higher volumes than those in the other solvents used in this study. This

Table 8 GC–MS analysis of BGP Ethanol extract. (- Not available) *(Boldface compounds have higher peak area %)*

S. No	Name of the compound	Molecular formula	Molecular weight	Retention time	Peak area (%)	Biological applications
1	2-Butylsulfanyl-1,1-diethoxy-2-butene	$C_{12}H_{24}O_2S$	232.38	3.593	4.34	-
2	Furfural	$C_5H_4O_2$	96.08	3.662	2.52	Nematicidal [134]; Insecticidal activity in plants and animals; biodegrading material [135]
3	Dimethyl sulfoxide	C_2H6OS	78.14	3.873	1.39	Anti-oxidant activity [136]
4	1,1-Diethoxy-3-heptanone	$C_{11}H_{22}O_3$	202.29	3.947	3.54	-
5	Isoamyl acetate	$C_7H_{14}O_2$	130.18	4.269	0.83	Anti-microbial agent [137]
6	2-Isopropoxyethanol	$C_5H_{12}O_2$	104.15	4.615	0.33	Hemolytic activity [138]
7	Benzoic acid, 3-methyl-, tert-butyldimethylsilyl ester	$C_{14}H_{22}O_2Si$	250.41	4.686	0.23	Anti-microbial activity [139]
8	2-Propenoic acid, 3-ethoxy-3-[(trimethylsilyl)oxy]-, ethyl ester	$C_{10}H_{20}O_4Si$	232.35	4.967	0.67	-
9	1,1-Diethoxy-3-methylbutane	$C_9H_{20}O_2$	160.25	5.701	1.34	Odor active compound [140]
10	Phenol	C_6H_5OH	94.11	6.287	0.46	Anti-microbial property [141]
11	Ethylene glycol diacetate	$C_6H_{10}O_4$	146.14	6.589	0.51	Biodiesel [142]
12	2-Ethylhexanol	$C_8H_{18}O$	130.23	7.513	0.77	Non-genotoxic carcinogen, peroxisome proliferator [143]
13	Acetophenone	C_8H_8O	120.15	8.503	0.78	Fumigant [144]; natural flavor compound [145]
14	1,1,3-Triethoxypropane	$C_9H_20O_3$	176.25	8.608	0.58	Odorant [146]
15	1,1,3-Triethoxybutane	$C_{10}H_{22}O_3$	190.28	9.258	1.56	Odorant [146]
16	Dodecane	$C_{12}H_{26}$	170.33	9.333	0.45	Skin irritant [147]
17	Nonanal	$C_9H_{18}O$	142.24	9.440	1.03	Anti-fungal activity [110]
18	Naphthalene	$C_{10}H_8$	128.17	11.615	**24.22**	Anti-alzheimer's agent [117]
19	Dodecane	$C_{12}H_{26}$	170.33	11.906	9.00	Skin irritant [147]
20	Decanal	$C_{10}H_{20}O$	156.26	12.045	1.18	Electroencephalographic activity [119], anti-microbial agent [120]
21	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	12.469	**6.91**	Anti-biofilm, Anti-virulence activity [121]
22	Nonanoic acid	$C_9H_{18}O_2$	158.24	13.492	0.52	Skin irritant [148]
23	2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone	$C_7H_{10}O_3$	142.15	14.400	0.76	Food flavoring agent [149]
24	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	$C_{12}H_{24}O_3$	216.32	16.160	0.97	-
25	1-Hexadecene	$C_{16}H_{32}$	224.42	16.598	1.11	Anti-microbial, anti-oxidant property [132]
26	Hexadecane	$C_{16}H_{34}$	226.44	16.787	8.80	Induces hyper Keratinization in tested rodents [109]
27	2,4,7,9-Tetramethyl-5-decyne-4,7-diol	$C_{14}H_{26}O_2$	226.35	16.941	0.79	Anti-foaming agent [150]
28	Heptadecane	$C_{17}H_{36}$	240.5	19.020	0.40	Anti-inflammation [151]; Anti-bactericidal [152]
29	1-Nonadecene	$C_{19}H_{38}$	266.5	20.974	1.28	Anti-oxidant [97]; Anti-bacterial [98]
30	Heptadecane	$C_{17}H_{36}$	240.5	21.137	4.47	Anti-inflammation [151]; Anti-bactericidal [152]
31	Octadecane	$C_{18}H_{38}$	254.5	23.137	0.37	Anti-fungal agent for plant and human pathogens [153]
32	1-Octadecene	$C_{18}H_{36}$	252.5	24.905	1.14	-

Table 8 (continued)

S. No	Name of the compound	Molecular formula	Molecular weight	Retention time	Peak area (%)	Biological applications
33	Heptadecane	$C_{17}H_{36}$	240.5	25.037	1.78	Anti-inflammation [151]; Anti-bactericidal [152]
34	Neophytadiene	$C_{20}H_{38}$	278.5	25.702	1.73	Anti-microbial property [126]; larvicidal activity [154]; anti-fungal activity [155]
35	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O4$	390.6	26.115	1.24	Anti-mutagenic activity [156]
36	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296.5	26.475	0.82	Anti-inflammatory, anti-oxidant [99]; anti-microbial [100]; larvicidal activity [101]
37	Methyl palmitate	$C_{17}H_{34}O_2$	270.5	27.258	0.93	Anti-radical activity [111], Anti-cancer activity [112]
38	1,1'-Sulfonylbis(3,5-dibromo-4-(2,3-dibromopropoxy) benzene)	$C_{18}H_{14}Br_8O_4S$	965.6	27.319	1.03	-
39	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	27.792	0.82	Plasticizer [157]
40	Palmitic acid	$C_{16}H_{32}O_2$	256.42	27.862	1.61	Anti-inflammatory activity [158] Anti-cancer activity [159]
41	Anthraquinone	$C_{14}H_8O_2$	208.21	28.291	0.80	Anti-proliferative activity [160, 161]
42	1-Hexadecanol	$C_{16}H_{34}O$	242.44	28.459	1.17	Anti-allergic and anti-histaminic activity [162]
43	Octadecane	$C_{18}H_{38}$	254.5	28.568	0.83	Anti-fungal agent for plant and human pathogens [153]
44	Methyl linolelaidate	$C_{19}H_{34}O_2$	294.5	30.087	0.26	Anti-oxidant [163]
45	Methyl petroselinate	$C_{19}H_{36}O_2$	296.5	30.194	0.52	Anti-microbial activity [164]
46	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296.5	30.363	0.79	Anti-inflammatory, anti-oxidant [99]; anti-microbial [100]; larvicidal activity [101]
47	Oleic acid	$C_{18}H_{34}O_2$	282.5	31.154	0.32	Anti-schistosomal activity [165]; anti-inflamatory [166]
48	7-Hexylicosane	$C_{26}H_{54}$	366.7	31.789	0.32	Anti-diabetic activity [167]
49	Hexadecanoic acid ((3E,7E)-(1S,2R)-2-hydroxy-1-hydroxymethyl-16-methyl-heptadeca-3,7-dienyl)-amide	$C_{35}H_{67}NO_3$	549.9	36.465	1.22	-
50	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	36.836	0.55	Anti-mutagenic activity [156]

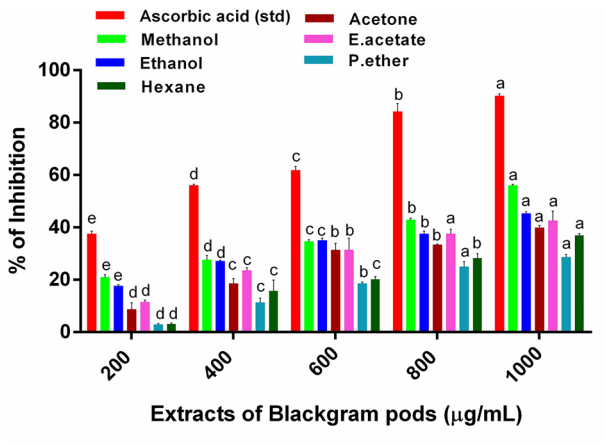
Table 9 The IC_{50} value of different extracts of black gram pods against DPPH and ABTS

IC_{50} (µg/mL)	Target	Positive Control	Methanol extract	Ethanol extract	Acetone extract	Ethyl acetate extract	Hexane extract	Petroleum ether extract
	DPPH	94.65	933.80	1163.43	1231.39	1145.65	1330.32	1634.12
	ABTS	205.65	976.28	1.057.57	1338.74	1292.32	1592.80	1413.70

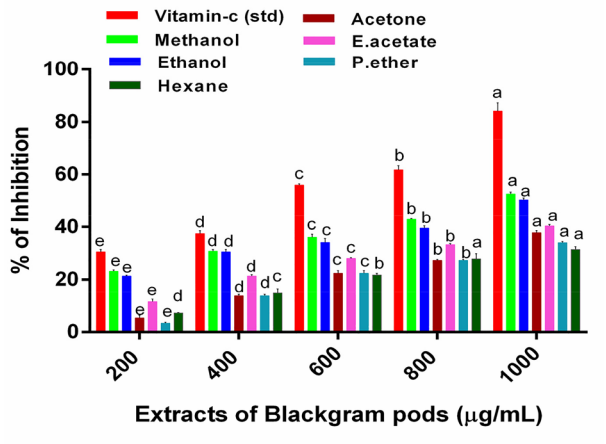
is due to extraction efficiency favoring the highly polar solvents and also the black gram pods contain high-level polar compounds that are capable of dissolving in high polar solvents [209]. These results are in accordance with the estimation of total phenol and total tannin content of the solvents extraction [210].

3.5.2 Total tannin content

As a result of this study, higher amounts of tannins were found in methanolic extracts (9.20 mg/TAE g) followed by ethanol (7.02 mg/TAE g), ethyl acetate (4.18 mg/TAE g), petroleum ether (3.99 mg/TAE g), acetone (3.42 mg/TAE g), and hexane (2.56 mg/TAE g) showing the range of results



a DPPH Radical scavenging activity of different extracts of black gram pods



b ABTS Radical scavenging activity of different extracts of black gram pods

Fig. 3 **a** DPPH Radical scavenging activity of different extracts of black gram pods. **b** ABTS Radical scavenging activity of different extracts of black gram pods

achieved in this investigation (Fig. 4d, Table 10) which was determined by the regression equation ($y = 0.0294x + 1.143$, $r^2 = 0.981$) of the tannic acid equivalent (TAE) given in Fig. 4c in our investigation. Even though both methanol and ethanol are polar solvents, they have different polarities as 0.762 and 0.654 respectively [32]. Tannin content was found higher in methanol than in ethanol because tannins have the ability to bind proteins leading to protein shrinkage and serving as astringents. Tannins have the capacity to directly destroy the bacterial cell wall, precipitate bacterial proteins, and cause bacterial death [211]. Tannins play a key role in the production of various nutraceuticals with different flavors [212]. Tannins are typically found in the bark of evergreen trees and owing to the combination of high polyphenols, they are a rich source of antibacterial and antioxidant properties [213]. Tannins are important chemicals with anti-phlogistic properties as well as cyclooxygenase-1 inhibitory activity and they may have anti-inflammatory benefits due to their anti-phlogistic characteristics [214–216]. The cyclooxygenase (COX) produces prostaglandins and lipoxygenase (LOX) with the help of arachidonic acid (AA) leads to the formation of leukotrienes and lipoxins. According to Cloutier and Guernsey [217], tannins induce arachidonic acid which induces prostaglandins through COX enzymes. COX 1 is present in all the cells that produce prostaglandins and affects blood flow and muscle proliferation. COX-2 enzyme, an essential component of the inflammatory cascade, is involved in the production of prostaglandins, which mediate pain and inflammation by increasing vascular permeability, allowing the extravasations of pro-inflammatory cells, proteins, and enzymes that mediate the reactions that lead to edema. Prostaglandins also make pain fibres more sensitive to mechanical and chemical stimuli [218, 219]. Tannins have a higher binding tendency with metallic ions and other molecules [220, 221]. Park et al. [222] reported that tannins modulate the inflammatory cytokines and inhibit the prostaglandins resulting in their anti-phlogistics property.

3.6 Assessment of antibacterial efficacy

3.6.1 Agar well diffusion method

The antibacterial potential was investigated with the various BGP extracts by the agar well diffusion method. These solvent extracts were compared to each other against tested microbes. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Aeromonas hydrophila* are the most widespread bacterial infections that cause severe mortality and morbidity in aquaculture [223–227]. Hemorrhagic septicemia, splenomegaly, abdominal distension, gill destruction, a clogged kidney, and a crumbly liver are all symptoms of these infections in *L. rohita.* Recently, *Klebsiella pneumoniae* was also identified as the pathogenic bacteria to fish particularly *Labeo rohita* and *Cyprinus carpio.* It has got various virulence genes that adopt bacterial pathogenesis [228, 229]. The bioactive compounds obtained with methanolic extracts were determined to be the most efficient antibacterial agents, according to our findings. Methanol had a much larger zone of inhibition than the other solvent extracts, whereas the other solvent extracts had small zones in a concentration-dependent manner due to polarity variations (Tables 11, 12, 13 14, 15, and 16). Efforts to develop plant-based antibacterial drugs have been stepped up in recent decades [230]. The antibacterial activity of the solvents rises as the polarity of the solvents increases [231, 232]. The most sensitive bacteria were *A. hydrophila*, *P. aeruginosa*, and *S. aureus*, whereas *K. pneumoniae* was the most resistant to all extracts tested

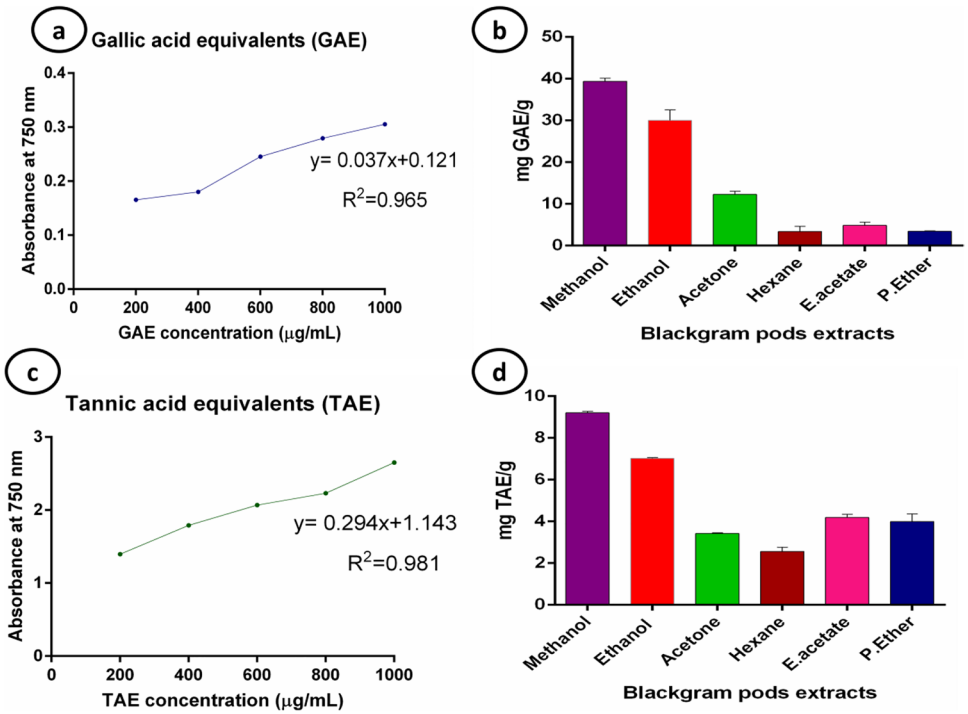


Fig. 4 Standard graph of **a)** gallic acid equivalents, **b)** quantitative analysis of the total phenolic content and standard graph of **c)** tannic acid equivalents, **d)** quantitative analysis of the total tannin content present in the various extracts of black gram pods

Table 10 Total phenol content and Total tannin content of the Black gram pod extracts

S. No	Solvent extracts	Total phenol content (mg/GAE g)	Total tannin content (mg/TAE g)
01	Methanol	39.37 ± 1.35	9.20 ± 0.11
02	Ethanol	30.01 ± 4.45	7.02 ± 0.05
03	Acetone	12.25 ± 1.30	3.42 ± 0.04
04	Ethyl acetate	4.83 ± 1.31	4.18 ± 0.26
05	Petroleum ether	3.43 ± 0.16	3.99 ± 0.63
06	Hexane	3.37 ± 2.15	2.56 ± 0.33

in this study. Gram-positive and Gram-negative bacteria differ in their susceptibility due to architectural variances specifically variances in membrane permeability [233, 234]. The Gram-positive bacteria cell's several layers of peptidoglycan create a resistant structure that prevents the bioactive compounds in the extracts from penetrating, but Gram-negative cell walls are made up of single or double layers of peptidoglycan, making them more susceptible to extracting secondary metabolites [235, 236]. Both gram-positive and gram-negative bacteria have a structure and composition that inhibits the drug from reaching the cytoplasmic membrane [237]. Major metabolites identified through GC–MS analysis of BGP extracts, such as butyronitrile, 3,7,11,15-tetramethyl-2-hexadecen-1-OL, phenol, 5-hydroxy methyl furfural, neophytadiene, isoamyl acetate, heptadecene, and 1-hexadecene could rupture the outer membrane and release lipopolysaccharides [238] through ion motive force [239] in the bacteria's membrane, these metabolites have the tendency to adhere the cytoplasmic membrane, causing membrane leakage, membrane integrity loss, and damage to the outer membrane vesicles (OMVs) [240]. DNA malfunction and the drug's inhibitory potential will be harmed as the electron density in DNA is altered. Plant phenolic compounds effectively metabolize the epithelium of harmful bacteria [241]. Methanol

Table 11 Antibacterial activity of the BGP methanol extract against tested microorganisms. Each result represents the mean ± standard error of the mean (n = 3), and different superscript letters indicate a significant difference between the groups ($p < 0.05$). "-" indicates no activity.

S. No	Zone of inhibition (mm)					
	Extracts	Control	250 (µg/mL)	500 (µg/mL)	750 (µg/mL)	1000 (µg/mL)
01	*Aeromonas hydrophila*	17.33 ± 1.52^{a}	-	10.16 ± 0.76^{d}	11.66 ± 1.52^{bc}	13.66 ± 1.52^{b}
02	*Pseudomonas aeruginosa*	20.50 ± 0.56^{a}	-	11.50 ± 1.80^{c}	14.66 ± 1.52^{b}	16.50 ± 0.52^{b}
03	*Staphylococcus aureus*	18.33 ± 3.21^{a}	-	10.83 ± 1.25^{b}	11.66 ± 1.52^{b}	14.33 ± 2.08^{b}
04	*Klebsiella pneumoniae*	18.33 ± 1.52^{a}	-	10.33 ± 1.04^{c}	11.33 ± 1.15^{c}	15.16 ± 1.32^{b}

Table 12 Antibacterial activity of the BGP ethanol extract against tested microorganisms. Each result represents the mean ± standard error of the mean ($n = 3$), and different superscript letters indicate a significant difference between the groups ($p < 0.05$). "-" indicates no activity.

S. No	Zone of inhibition (mm)					
	Extracts	Control	250 (µg/mL)	500 (µg/mL)	750 (µg/mL)	1000 (µg/mL)
01	*Aeromonas hydrophila*	17.19 ± 1.04^{a}	-	8.35 ± 1.32^{c}	11.33 ± 0.57^{b}	15.33 ± 1.52^{a}
02	*Pseudomonas aeruginosa*	19.33 ± 1.15^{a}	-	9.66 ± 0.57^{d}	12.83 ± 0.76^{c}	15.59 ± 0.86^{b}
03	*Staphylococcus aureus*	19.29 ± 2.64^{a}	-	8.52 ± 0.86^{d}	11.5 ± 0.56^{c}	16.52 ± 1.32^{b}
04	*Klebsiella pneumoniae*	19.16 ± 1.04^{a}	-	8.33 ± 1.52^{d}	11.83 ± 0.76^{c}	15.16 ± 1.60^{b}

Table 13 Antibacterial activity of the BGP acetone extract against tested microorganisms. Each result represents the mean ± standard error of the mean ($n = 3$), and different superscript letters indicate a significant difference between the groups ($p < 0.05$). "-" indicates no activity.

S. No	Zone of inhibition (mm)					
	Extracts	Control	250 (µg/mL)	500 (µg/mL)	750 (µg/mL)	1000 (µg/mL)
01	*Aeromonas hydrophila*	17.16 ± 1.04^{a}	-	9.5 ± 0.86^{c}	13.29 ± 1.32^{b}	16.52 ± 1.60^{a}
02	*Pseudomonas aeruginosa*	20.33 ± 2.08^{a}	-	10.16 ± 1.04^{d}	13.04 ± 1.04^{c}	16.66 ± 0.57^{b}
03	*Staphylococcus aureus*	18.66 ± 1.60^{a}	-	9.83 ± 0.76^{d}	13.33 ± 1.15^{c}	16.5 ± 0.85^{b}
04	*Klebsiella pneumoniae*	18.16 ± 0.76^{a}	-	9.83 ± 0.76^{d}	13.5 ± 0.52^{c}	15.83 ± 0.76^{b}

Table 14 Antibacterial activity of the BGP ethyl acetate extract against tested microorganisms. Each result represents the mean ± standard error of the mean ($n = 3$), and different superscript letters indicate a significant difference between the groups ($p < 0.05$). "-" indicates no activity.

S. No	Zone of inhibition (mm)					
	Extracts	Control	250 (µg/mL)	500 (µg/mL)	750 (µg/mL)	1000 (µg/mL)
01	*Aeromonas hydrophila*	19.33 ± 0.57^{a}	-	-	12.16 ± 0.76^{c}	15.5 ± 0.56^{b}
02	*Pseudomonas aeruginosa*	20.5 ± 1.32^{a}	-	-	12.83 ± 1.25^{c}	16.5 ± 1.32^{b}
03	*Staphylococcus aureus*	20.33 ± 1.32^{a}	-	-	12.83 ± 1.25^{c}	16.16 ± 0.76^{b}
04	*Klebsiella pneumoniae*	19.66 ± 0.57^{a}	-	-	12.5 ± 1.32^{c}	16.16 ± 1.04^{b}

Table 15 Antibacterial activity of the BGP hexane extract against tested microorganisms. Each result represents the mean ± standard error of the mean ($n = 3$), and different superscript letters indicate a significant difference between the groups ($p < 0.05$). "-" indicates no activity.

S. No	Zone of inhibition (mm)					
	Extracts	Control	250 (µg/mL)	500 (µg/mL)	750 (µg/mL)	1000 (µg/mL)
01	*Aeromonas hydrophila*	19.16 ± 0.76^{a}	-	-	11.83 ± 1.04^{c}	14.5 ± 0.56^{b}
02	*Pseudomonas aeruginosa*	19.5 ± 0.86^{a}	-	-	13.5 ± 0.56^{c}	15.33 ± 1.52^{b}
03	*Staphylococcus aureus*	20.33 ± 1.52^{a}	-	-	12.5 ± 0.86^{c}	15.16 ± 0.76^{b}
04	*Klebsiella pneumoniae*	19.16 ± 0.76^{a}	-	-	12.16 ± 0.76^{c}	15.16 ± 0.76^{b}

Table 16 Antibacterial activity of the BGP petroleum ether extract against tested microorganisms. Each result represents the mean ± standard error of the mean ($n = 3$), and different superscript letters indicate a significant difference between the groups ($p < 0.05$). "-" indicates no activity.

S. No	Zone of inhibition (mm)					
	Extracts	Control	250 (µg/mL)	500 (µg/mL)	750 (µg/mL)	1000 (µg/mL)
01	*Aeromonas hydrophila*	18.56 ± 1.52^{a}	-	-	13.35 ± 1.32^{c}	15.83 ± 0.76^{b}
02	*Pseudomonas aeruginosa*	19.52 ± 1.32^{a}	-	-	13.83 ± 0.76^{c}	16.66 ± 1.32^{b}
03	*Staphylococcus aureus*	20.16 ± 1.25^{a}	-	-	14.57 ± 0.76^{c}	16.5 ± 0.56^{b}
04	*Klebsiella pneumoniae*	18.66 ± 1.52^{a}	-	-	13.03 ± 0.76^{c}	17.56 ± 0.5^{b}

extract has the strongest antibacterial efficacy against all the microorganisms tested, which is due to the extraction of soluble metabolites by the solvent [242, 243].

3.6.2 Determination of minimum inhibitory concentration

Identification of minimum inhibitory concentrations (MIC) of various BGP extracts using the resazurin dye technique is shown in Table 17. Methanol and ethanol extracts had potential MIC values at 250 µg/mL against all the investigated bacteria, but the other extracts possessed bacterial inhibition at 500 µg/mL. Resazurin reduction could be accomplished by live bacterial cells. The extracts' inhibitory activity was aided by the incorporation of long-chain free fatty acids like oleic acid, palmitic acid, and linolenic acid. Enoyl-acyl reductase is a bacterial carrier protein involved in fatty acid production [244]. But the extracts have the tendency to inhibit bacterial growth by reducing the carrier protein reductase formation [245–247]. The presence of unsaturated fatty acids in the BGP extracts was confirmed in the GC–MS analysis in this investigation, which yielded similar results. The polarity of the molecule is important because it determines how it enters the organism's membrane, causing growth disruption [78]. The extracts' antibacterial action is dominated by phenols, which are followed by aldehydes, ketones, alcohols, ethers, and hydrocarbons [248]. The anti-microbial property of the BGP extracts is based on the metabolites present in the extracts able to bind the membrane of the bacterial cell through hydrogen bonding and hydrophobic interactions [249]. Thus, it alters the membrane permeability and allows the metabolites and molecules present in the extracts to inside the bacterial cell [250]. Because Gram-negative bacteria have a thicker cell wall than Gram-positive bacteria, they are more resistant to crude extracts, oils, and their metabolites [251]. The transmembrane hydrophilic protein called porin doesn't allow the hydrophobic metabolites [252]. Another possible aspect of the bacterial growth inhibition is inhibiting the ATPase activity, in that the electron transport chain creates protons which required for the ATP synthesis. These protons pass through the membrane by efflux pump to the cytoplasm and maintain its pH [253]. The metabolites like 1-nonadecane [254], heptadecene [255], isoamyl acetate [256], and phenol [257] in both polar and non-polar extracts of the black gram pods disrupt the proton motive force and cause the depletion of the ATP synthesis by damaging the mitochondria [258]. Phenols operate as a proton donor/acceptor, which could contribute to antimicrobial action [259, 260]. The existence of different bioactive components in BGP extracts and their various fractions, such as saponins, phenolics, and flavonoids, could contribute to the antibacterial activity's diversity [261].

3.6.3 Effects on growth of the various BGP extracts on microbial cultures

The influence of different extracts on the growth of *A. hydrophila*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* is depicted in Figs. 5, 6, 7, and 8 based on the obtained data. In vitro level of sensitivity or resistance of various bacterial strains to administered drug is defined by the minimal inhibitory concentration (MIC). The ability to accurately measure MIC has a substantial impact on the therapeutic method chosen, which impacts the efficacy of infection treatment [262]. And sub-MIC is half of the MIC that is used to determine the inhibitory potential of the drug at ½ minimal inhibitory concentrations [263]. Several studies were conducted to evaluate the antibacterial efficacy of the extracts at both MIC and sub-MIC levels [264–267]. Similarly, in this study, the efficacy of the black gram pod extracts was evaluated on bacterial growth analysis at determined MIC and sub-MIC (½ MIC) in Table 17. The results revealed that at sub-MIC level, the BGP extracts did not show any inhibitory activity in all the prepared extracts. However, at the MIC level, growth was completely stopped. This study shows that several BGP extracts can suppress the growth of the examined microbial cultures at the MIC level, but not at the Sub-MIC level. The hydrophobic compounds in the extracts were in direct contact with the studied microorganisms, causing the inhibitory effect [268]. The MIC value found in this study corresponds

Table 17 Minimum inhibitory concentration (MIC) and sub-minimal inhibitory concentration (sub-MIC) values of the Black gram pod extracts against the tested microorganisms

Solvent used for Black gram pods extracts	*A. hydrophila*		*P. aeruginosa*		*S. aureus*		*K. pneumoniae*	
	MIC (µg/mL)	Sub-MIC (µg/mL)	MIC (µg/mL)	Sub-MIC (µg/mL)	MIC (µg/mL)	Sub-MIC (µg/mL)	MIC (µg/mL)	Sub-MIC (µg/mL)
Methanol	250	125	250	125	250	125	250	125
Ethanol	250	125	250	125	250	125	500	250
Acetone	500	250	250	125	250	125	500	250
Ethyl acetate	500	250	500	250	500	250	500	250
Hexane	500	250	500	250	500	250	500	250
Petroleum Ether	500	250	500	250	500	250	500	250

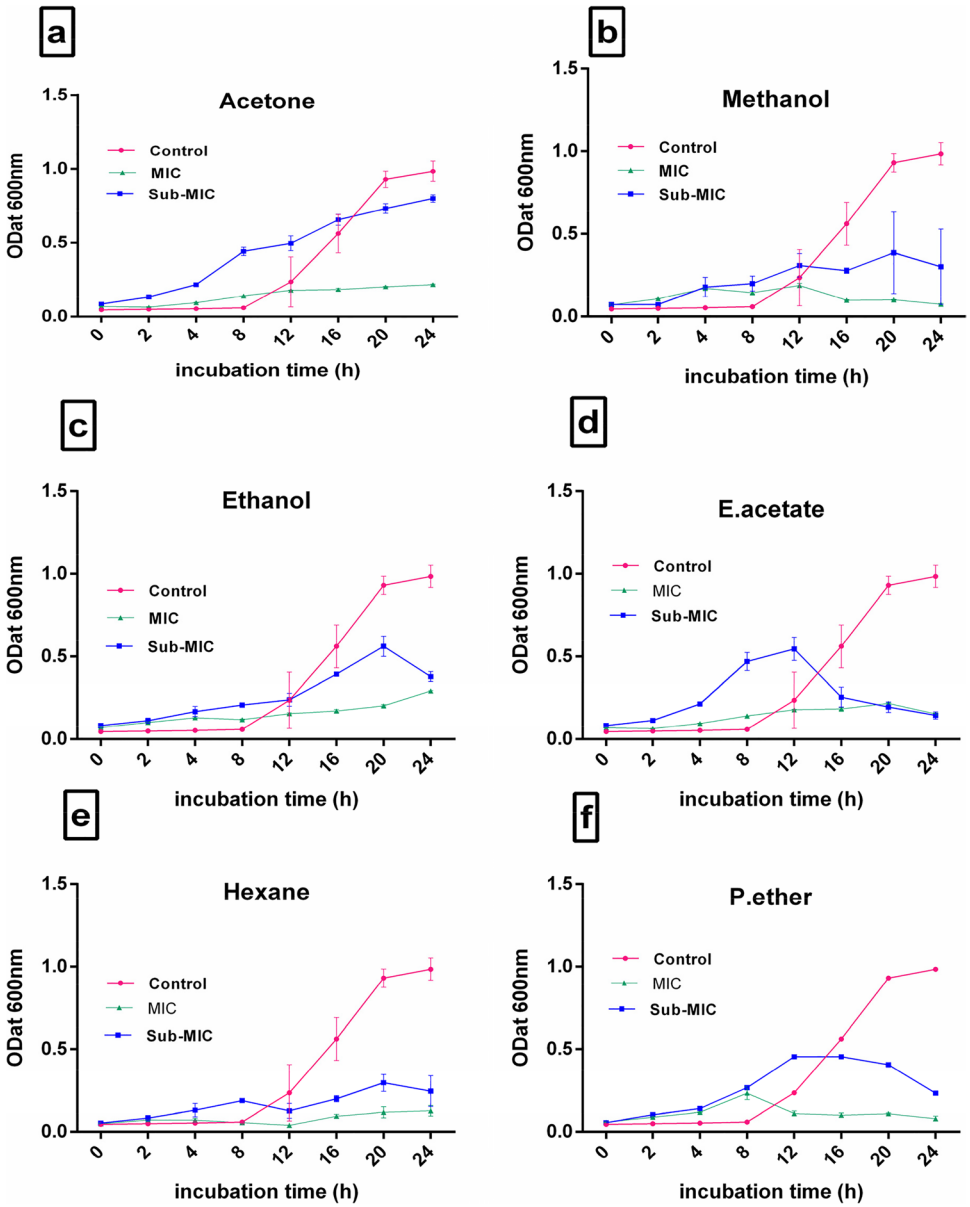


Fig. 5 Growth curves of *Aeromonas hydrophila* under the influence of various extracts of black gram pods (**a**-acetone, **b**-methanol, **c**-ethanol, **d**-ethyl acetate, **e**-hexane and **f**-petroleum ether)

to the bacterial growth curve analysis, which revealed inhibition in both polar and non-polar crude extracts of the BGP [269]. Bacterial cell membranes are involved in various functions like adhesion, conductivity, and signaling. When the cell is exposed to the drugs/antibiotics, it generates free radicals and causes damage to the membrane [270]. Simultaneously, when the membrane permeability of the cell increases, it leads to the reduction of ion gradients, loss of proteins involved in various mechanisms, and inhibition of cellular metabolism [271]. Esters of phenolic acids like hydroxybenzoic, hydroxyphenylacetic, and hydroxycinnamic acids have potential antioxidant activity and are proportionate to the number of hydroxyl groups in a molecule that is hampered by hydrophobic interactions from their carboxyl group. While comparing to the hydroxybenzoic acid, both the hydroxyphenylacetic and hydroxycinnamic acids possess higher proton-donating ability leading to an increase in the antioxidant potential [272]. Generally, the electron donors driven proton motive force leads to mitochondrial ROS [273].

3.6.4 Inhibition of biofilm formation and development by BGP extracts

Solvents play an important role in extracting a certain component from plants; the type of compound retrieved from the plants will be determined by the composition of the solvent. As a result, a variety of solvents were utilized to extract bioactive compounds from the plants [274]. When compared to other solvents, the extraction done in alcoholic solvents

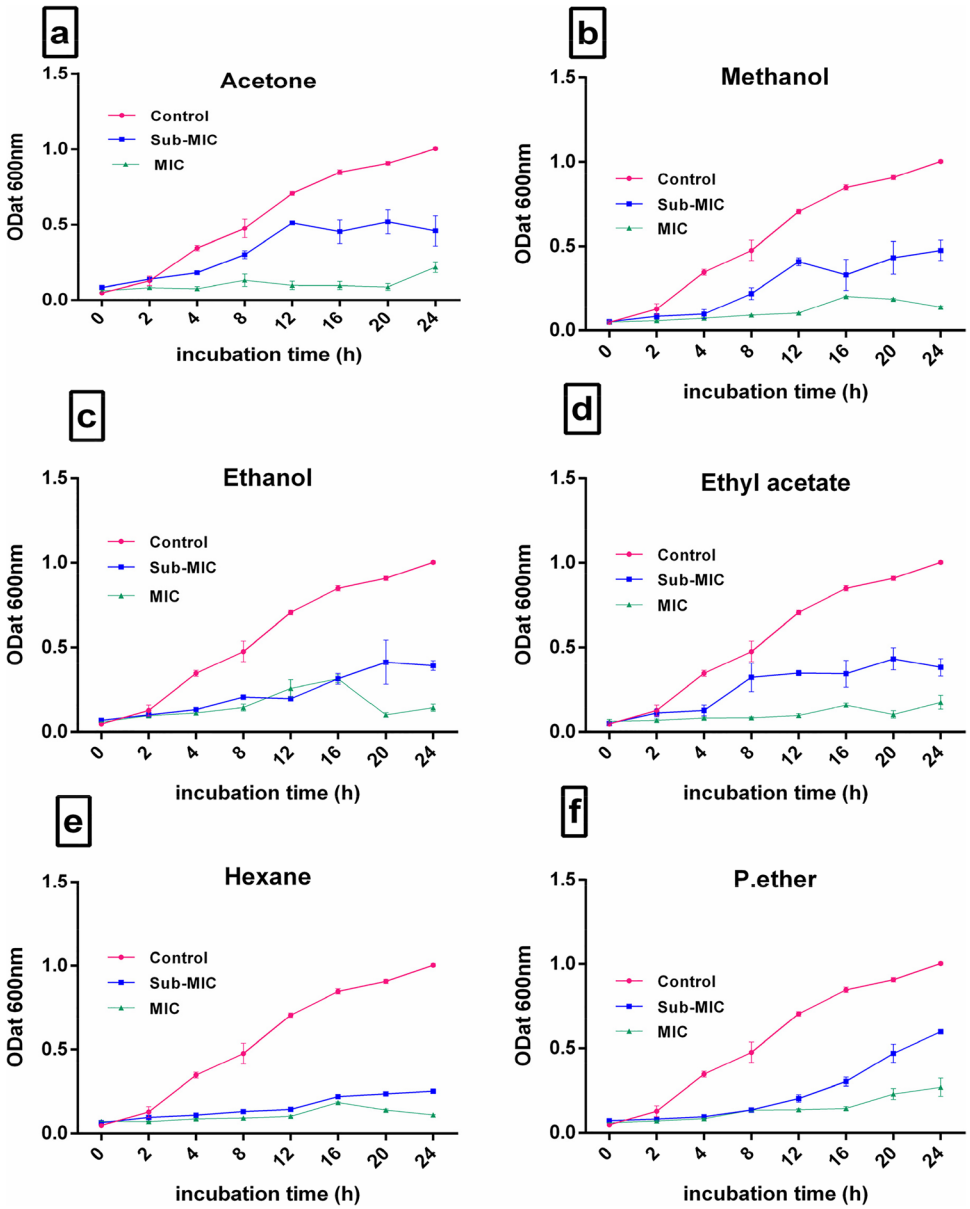


Fig. 6 Growth curves of *Pseudomonas aeruginosa* under the influence of various extracts of black gram pods (**a-**acetone**, b-**methanol, **c-**ethanol, **d-**ethyl acetate, **e-**hexane, and **f-**petroleum ether)

yielded highly encouraging results. Extraction of the same type of plants in different solvents has distinct sorts of activity against biofilm [275]. The bacterial community is embedded in the polymer matrix and associates various risk problems in human life due to the reduced susceptibility of the tested bacteria in the form of biofilms [276]. Several studies had failed to confirm the susceptibility of antimicrobial agents in the form of biofilm. This study was conducted to identify a susceptible range of various BGP extracts as an antibiofilm agent; 1000 µg/mL showed better results by inhibiting the biofilm formation (Fig. 9a–d). Antibacterial drugs have the ability to suppress or destroy biofilm formation hold promise for minimising microbial colonisation of surfaces, epithelial mucosa, and matrix formation [277]. Compared to polar, non-polar solvents possessed lower anti-biofilm activity because of interference by the extracts on the ability of these microbes to adhere to the surfaces. Plant-based extracts have been found to disrupt bacteria biofilm development by mechanisms like disrupting microbial membrane structures and blocking peptidoglycan synthesis [278]. *Pseudomonas aeruginosa* produces a quorum-sensing regulating virulence factors such as protease, elastase, and chitinase [279]. These enzymes affect the host cell proteins and induce the bacterial growth and formation of biofilm. The extracts of BGP inhibit the production of enzymes on a dose-dependent manner and reduce the biofilm formation [280]. In *Aeromonas hydrophila*, the exo-proteases and exo-polysaccharide production by ahyR1. The BGP extracts have the tendency to act on the ahyR1 and cause the production of C4-HSL. And it may block the quorum sensing [281] by inhibiting the proton motive force linked to the motility activity-dependent efflux

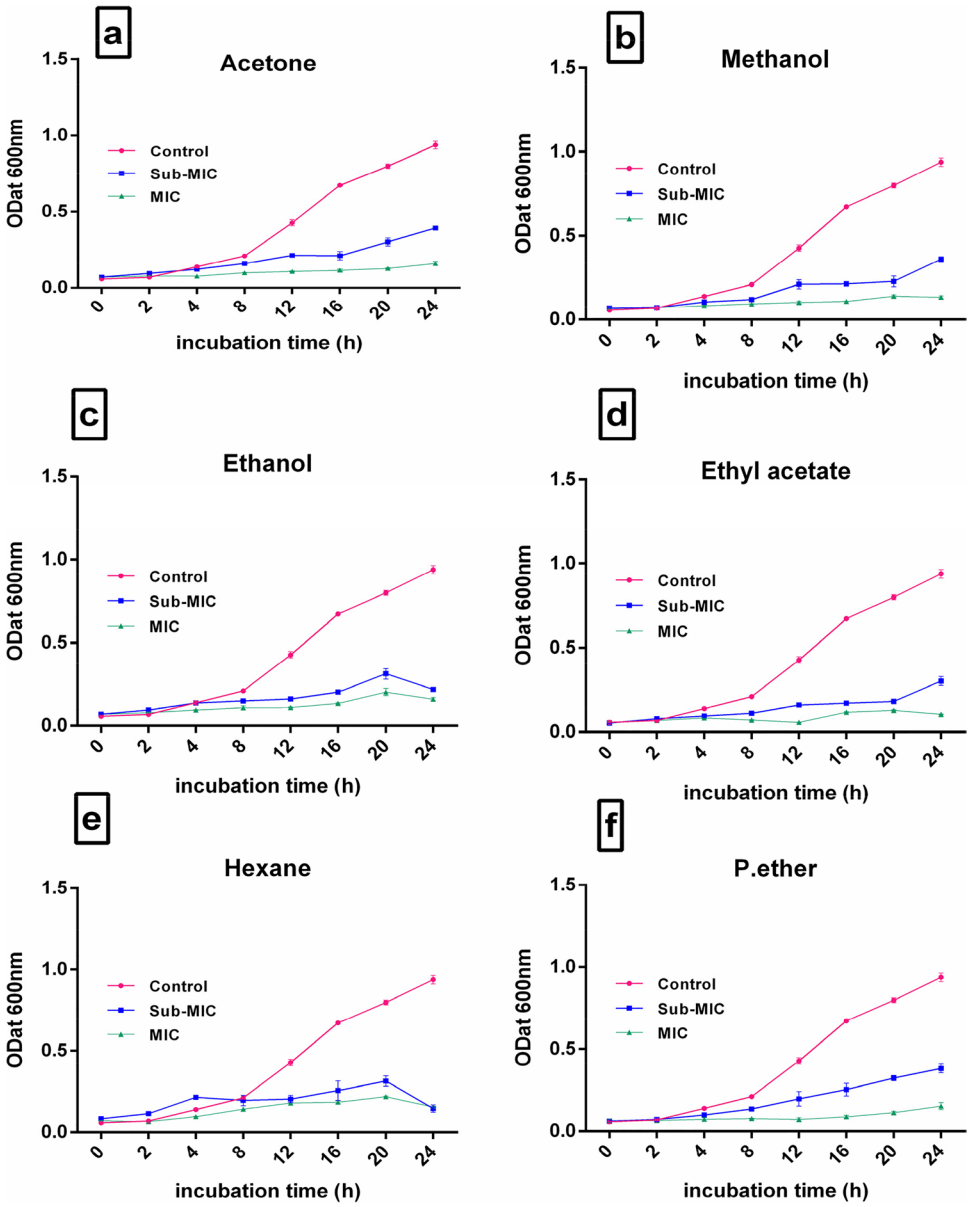


Fig. 7 Growth curves of *Klebsiella pneumoniae under* the influence of various extracts of black gram pods (**a-**acetone, b-methanol, **c-**ethanol, **d-**ethyl acetate, **d-**acetone, **e-**hexane and **f-**petroleum ether)

pump in the biofilm formation [282]. Similarly in *Klebsiella pneumoniae,* AHL (acyl homoserinelactones) an autoinducer for the production of exopolysaccharides that generates virulence factors for the formation of biofilm, the compounds in the BGP extracts block the biosynthesis of autoinducers by degrading the specific enzymes or preventing its interactions with the receptors [283]. Staphyloxanthin is a pigment found in *Staphylococcus aureus* that works as a redox toxin, stimulating the production of biofilm matrix by increasing extracellular DNA [284]. The biofilm inhibitory activity of metabolites has been linked to modulation of bacterial cell–cell communication [285], interference with surface hydrophobicity, motility, and charge [286], and dysregulation of biofilm-related genes [287]. We anticipated that metabolites of BGP extracts reduce staphyloxanthin synthesis, resulting in a reduction of biofilm formation in *S. aureus*. Moreover, when the concentration of the BGP extracts increases; the inhibiting potential was also increased. This could be a promising strategy to minimize the microbial colonization of intestinal mucosa, which leads to infections; consequently, this study proved the inhibitory potential of the BGP extracts against aquatic pathogens like *A. hydrophila*, *K. pneumoniae, S. aureus*, and *P. aeruginosa*.

4 Conclusion

According to a qualitative phytochemical study, plant-based extracts are advantageous due to the presence of phytochemicals such as phenols, tannins, saponins, flavonoids, and alkaloids. This study revealed the identification of bioactive metabolites

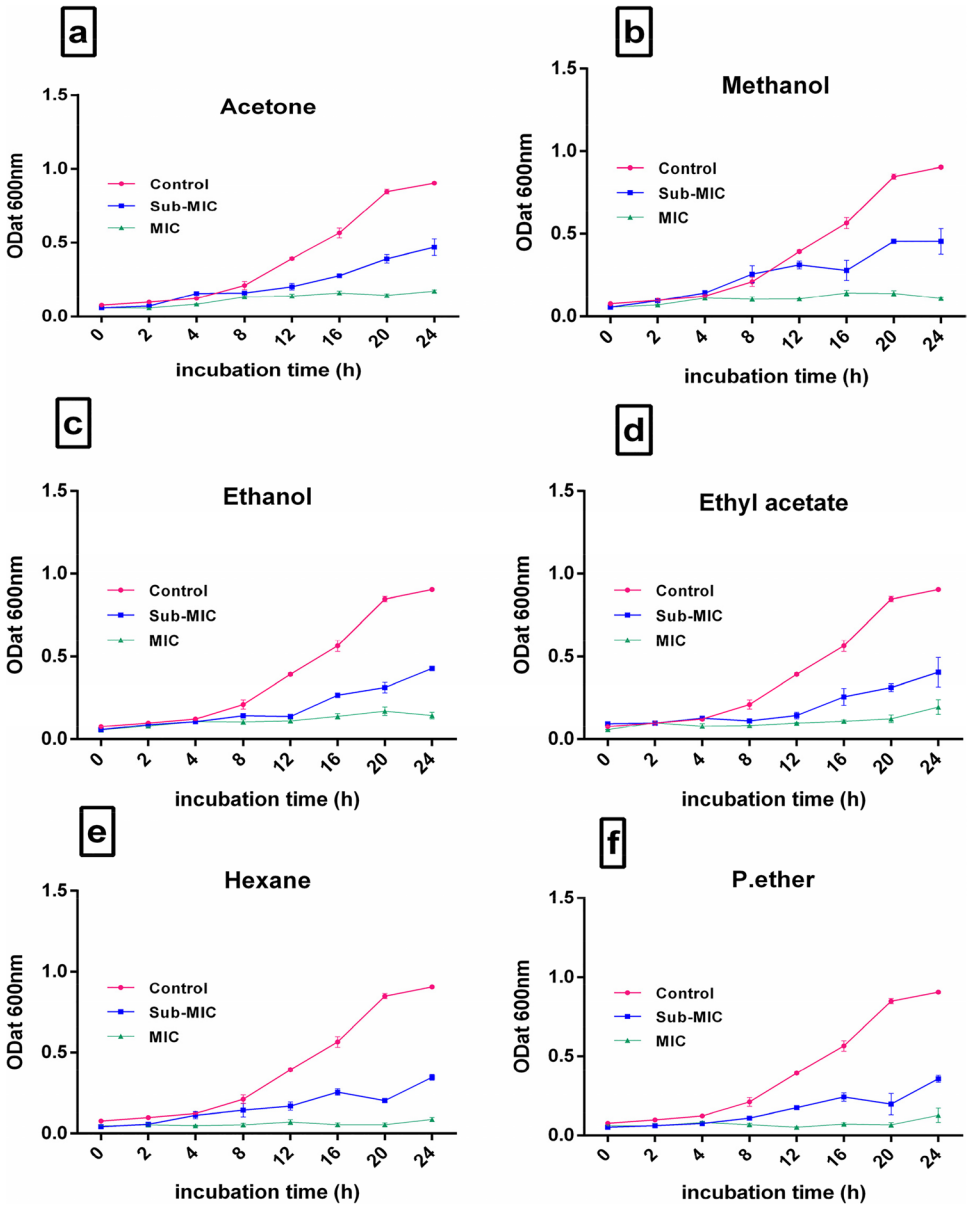


Fig. 8 Growth curves of *Staphylococcus aureus* under the influence of various extracts of black gram pods (**a-**acetone**, b-**methanol, **c-**ethanol, **d-**ethyl acetate, **e-**hexane and **f-**petroleum ether)

from the agricultural waste of black gram pods. FT-IR and GC–MS were used to identify and confirm functional groups and bioactive metabolites present in the black gram pods. The presence of key metabolites such as tetratetracontane, butyronitrile, cholesterol, tetracontane, 2,2-difluorocycloheptan-1-one, 3,7,11,15-tetramethyl-2-hexadecen-1-OL, and diacetone alcohol in BGP extract suggests that it has a higher antioxidant, agonist to treat cancer, and antibacterial potential. Methanolic extract was found to be a better antioxidant and antibacterial agent than other extracts against the tested microorganisms. The study findings showed a wealth of information about agricultural waste BGP which can be used to combat pathogens in aquatic disease control. Furthermore, this groundbreaking research promotes the use of biowaste as a possible conversion of drug sources in the pharmaceutical industry.

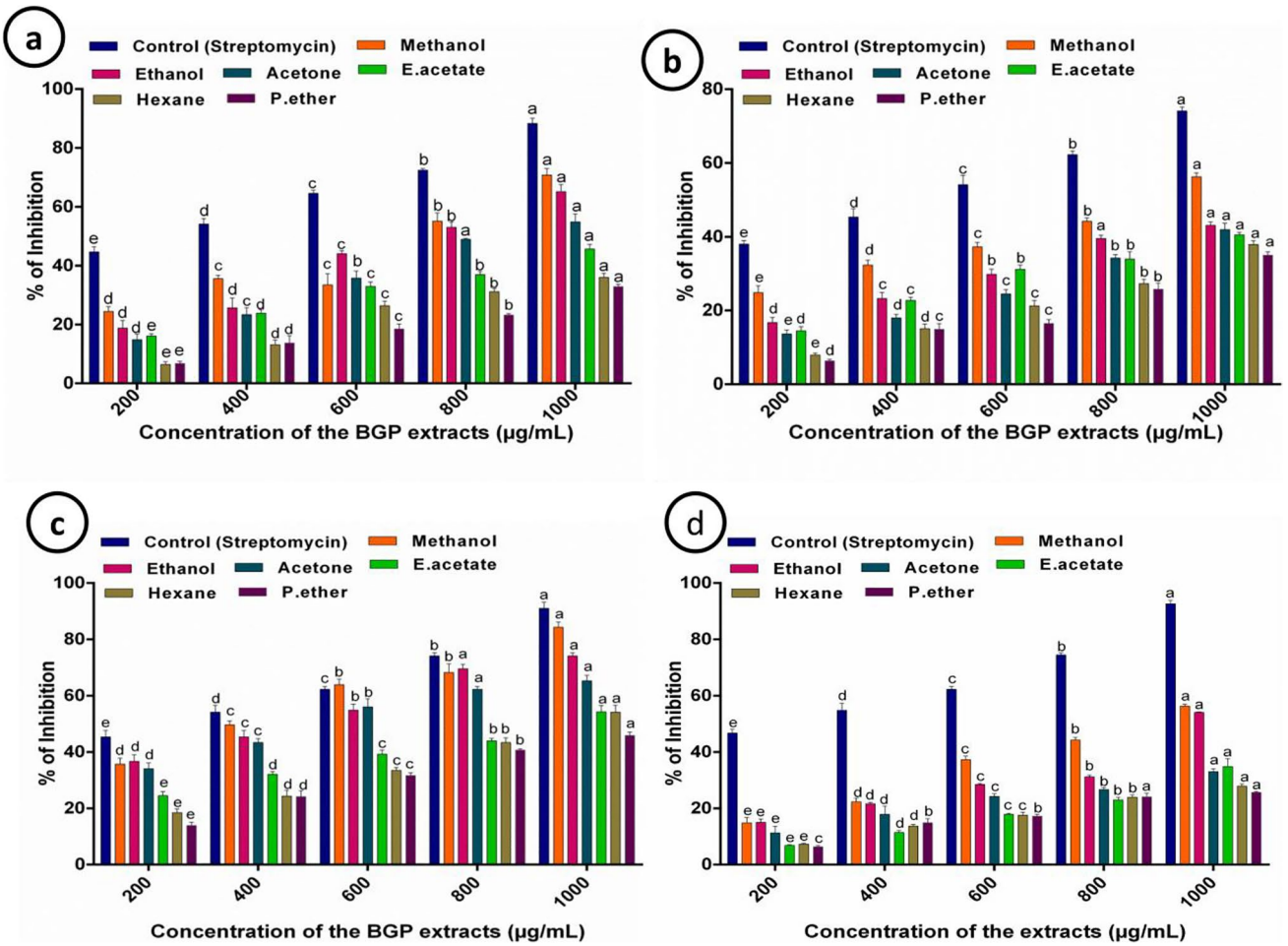


Fig. 9 Inhibition of biofilm formation of various extracts of BGP against **a** *A. hydrophila*, **b** *K. pneumoniae*, **c** *S. aureus*, **d** *P. aeruginosa*

Author contribution Manikandan Arumugam: investigation, methodology, formal analysis, writing—original draft, writing—review and editing. Dinesh Babu Manikandan: investigation, resources, data curation. Sujitha Mohan: resources, data curation. Arun Sridhar: formal analysis, data curation, writing—review and editing. Sudharshini Jayaraman: resources, data curation. Srinivasan Veeran: formal analysis, data curation. Thirumurugan Ramasamy: conceptualization, project administration, supervision, validation, visualization, writing—review and editing.

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Declarations

Conflict of interest The authors declare no competing interests.

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