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EXPERIMENTAL PAPER

Phytochemical profiling, assessment of *in-vitro* antimicrobial and antioxidant activities of different parts of Indian gooseberry (*Phyllanthus emblica* Linn.)

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Summary

Introduction: The Indian gooseberry, also known as Amla, or *Phyllanthus emblica* L., is well known for its diverse phytochemical makeup and therapeutic uses.

Objective: The objective of the study was to investigate the phytochemical profiles, antibacterial properties, and *in-vitro* antioxidant activity of various parts of *P. emblica*, including leaves, fruits, and seeds.

Methods: Maceration extraction was performed using ethanol, petroleum ether, acetone and sterile distilled water. The phytochemicals in the extracts were analysed both qualitatively and quantitatively using standard laboratory techniques. The antioxidant activity was measured by DPPH and ABTS assays while antimicrobial activity was evaluated by antibacterial and antifungal *in-vitro* bioassays. Gram-negative and gram-positive microorganisms were tested for antibacterial activity utilizing the agar well diffusion method. The antifungal activity against three fungus strains: A*spergillus* spp., *Fusarium* spp. and *Mucor* spp. was assessed by diffusion method.

Results: All three parts of *P. emblica* are rich in flavonoids, alkaloids, tannins, phenols, vitamin C, carboxylic acids, reducing sugars, coumarins and quinones. Ethanolic leaf extracts recorded the highest flavonoid (32.24±1.87 μg QE/g) and tannin content (505.26±1.67 μg TAE/g), while aqueous fruit extracts had significant phenolic content (24.83±1.73 μg GAE/g). Alkaloids were most abundant in fruits (56.88±2.88 mg/g). Both assays revealed the highest radical scavenging potential in fruit ethanolic extract (IC_{50} value for DPPH assay = 130.649±0.918 mg/ml, IC_{50} value for ABTS assay = 266.178±0.777 mg/ml). Ethanolic fruit extracts exhibited the strongest antibacterial effects, with inhibition zones of 39.67±0.58 mm against *S. aureus*, 27.00±0.05 mm against *E. coli*, and 35.33±0.57 mm against *P. aeruginosa*. Antifungal activity was most pronounced against *Fusarium* spp., with growth inhibition percentage of 56.49% in ethanolic leaf extracts.

Conclusions: The study emphasizes potential of *P. emblica* as a natural source of antioxidants and antibacterial agents while defending its conventional medical use.

Key words: ***Phyllanthus emblica, antioxidant, antimicrobial, phytochemicals, medicinal plants***

Sowa kluczowe: ***Phyllanthus emblica, diamante przeciwutleniające i przeciwdrobnoustrojowe, rośliny lecznicze***

INTRODUCTION

Medicinal plants have been an integral part of traditional healthcare systems in many societies since prehistoric times. The use of herbal medicine is increasingly gaining global recognition for its role in promoting health and safety, primarily due to its lower risk of adverse effects [1]. Medicinal plants produce a wide array of bioactive compounds of multiple purposes, including action against microbial pathogens, herbivorous insects, and environmental stressors [2]. Phytochemical studies have identified numerous plant-derived compounds, some with well-established pharmacological effects and others with potential but yet to be fully explored biological functions [3, 4].

Because of its many different bioactive components, *Phyllanthus emblica*, often known as Indian gooseberry or amla, has been utilized extensively in Ayurvedic, Unani, and Tibetan medicine [5]. It contains a lot of phytochemicals, predominantly including flavonoids, tannins, alkaloids, phenols, and vitamin C, which contribute to its potent antioxidant, antimicrobial, hepatoprotective, and anticancer activities [6, 7]. The therapeutic potential of *P. emblica* is widely recognized for preventing oxidative damage, enhancing immune responses, and counteracting microbials [3].

Antioxidants have attracted significant interest for their essential role in neutralizing free radicals, consequently lowering ailments linked to oxidative stress, including cancer, neurological diseases, and cardiovascular diseases [8]. *P. emblica* exhibits strong antioxidant properties, primarily attributed to its high ascorbic acid and polyphenol content [9]. Additionally, its antimicrobial effects have been well documented, with various extracts showing inhibition against bacterial and fungal pathogens [6].

This study aims to discover the phytochemical profile of *P. emblica* and validate its antioxidant and antimicrobial efficacy through *in-vitro* assays, contributing to potential pharmaceutical applications.

MATERIALS AND METHODS

Plant materials

Various parts of the *P. emblica* plant, including its leaves, fruits, and seeds (fig. 1, 2 and 3, respectively) were collected in November 2023 from the Jaffna district. The specimen was placed in the National Herbarium of the Department of National Botanic Gardens, Peradeniya, after being botanically authenticated. The voucher specimen number for the deposited specimen is PDA00109618.

Figure 1.

Leaves of *P. emblica*

Figure 2

Fruits of *P. emblica*

Figure 3.

Seeds of *P. emblica*

Preparation of plant materials

After collection, the leaves, seeds, and fruits were properly cleaned with tap water to get rid of any contaminants. After air dry, they were further dried in oven for five days at 45°C. Once completely dried, they were finely ground into a powder using electric grinder and sieved through an 80-mesh sieve. For subsequent analysis, the powdered material was kept in airtight containers.

Making aqueous, acetone, petroleum ether, and ethanolic extracts

Powdered plant material in a quantity of 40 g was placed in separate conical flasks, to which 200 millilitres of ethanol, petroleum ether, acetone and sterile distilled water were added (Avon Pharmo Chem (Pvt) Ltd). The flasks were securely covered and placed in an orbital shaker for occasional shaking at 100 rpm. Following extraction, Whatman No. 1 filter paper was used to filter the mixture. A rotary evaporator (Buchi) was used to concentrate some of the filtrate, and the remaining material was refrigerated at 4°C for later use.

Qualitative phytochemical analysis

In order to identify the presence of 25 distinct phytochemicals, standard laboratory procedures were used to screen phytochemicals of the ethanol, petroleum ether, and aqueous extracts of each powder (leaf, fruit, and seed) (Table 1).

Quantitative phytochemical analysis

Total concentration of phytochemicals was evaluated using standard laboratory protocols (tab. 2) for quantitative analysis of phytochemicals from different extracts (ethanolic, petroleum ether, and aqueous) of leaves, fruits and seeds of *P. emblica*.

Assessment of antioxidant activity of leaf, fruit and seed of *P. emblica* in their ethanolic extracts

The antioxidant ability was assessed using DPPH and ABTS free radical scavenging assays and IC_{50} values were computed to compare the effectiveness of the extracts.

Antioxidant activity by DPPH free radical scavenging method

Total free radical scavenging capacity of *P. emblica* extracts (leaves, fruits, and seeds) was assessed using DPPH radical which has an absorption maximum at 515 nm. A 2.4 mg DPPH solution (Sigma Aldrich Chemical Company, St Louis, MO, USA) in 100 ml ethanol was mixed with 5 μl of ethanolic extract at concentrations of 100–500 mg/ml. After shaking, the mixture was kept at room temperature for half an hour in the dark. Absorbance was measured at 515 nm, with blank (DPPH solution without antioxidant) also recorded. All samples were assayed in triplicates and the average was used for analysis (fig. 4).

DPPH scavenging ability (%) was calculated as:

$$\text{DPPH Scavenged (\%)} = \frac{(A_B - A_A)}{A_B} \times 100,$$

where antioxidant absorbance at 30 minutes is denoted by $\mathbf{A_A}$ and blank absorbance at 0 minutes by $\mathbf{A_B}$.

By graphing the percentage inhibition against concentration, the extract concentration needed to scavenge 50% of the DPPH radicals (IC_{50}) was determined.

Table 1.

Tests of qualitative analysis of phytochemicals

Phytochemicals	Tests	Expected observations
Flavonoids	Alkaline reagent test	Disappearance of formed yellow colour
Phenols	Ferric chloride test	Formation of blue colour
Tannins	Ferric chloride test	Formation of black or brownish blue
Alkaloids	Wagner's test	Formation of reddish-brown precipitate
Terpenoids	Chloroform test	Formation of reddish-brown colouration at the interface
Diterpenes	Copper acetate test	Emerald green colour
Saponins	Foam test	Development of stable foam
Cardiac glycosides	Keller-Kiliani test	Brown ring at the interface
Anthocyanins	[24]	Purplish blue colour solution
Steroids	Salkowski test	Reddish brown colour appearance at the lower layer
Phytosterols	Salkowski test	Formation of brown colour ring at the interface
Anthraquinones	[25]	Appearance of blood red colour
Quinones	[26]	Appearance of blue, green or red colour
Coumarins	[27]	Development of yellow colour
Carboxylic acids	Sodium bicarbonate test	Formation of effervescence
Reducing sugars	Fehling test	Red colour precipitation
Xanthoproteins	Xanthoproteic test	Dark yellow or orange solution
Proteins and amino acids	Xanthoproteic test	Yellow colour solution
Vitamin C	[28]	Disappearance of violet colour
Lignins	Maule colour test	Red colouration
Carotenoids	[29]	Blue colour interface
Resins	Acetic anhydride test	Orange to yellow colour solution
Phlobatannins	HCl test	Red precipitate
Carbohydrates	Molisch's test	Violet to reddish colour solution
Fixed oils and fats	Spot test	Oil stain between 2 filter papers

Determination of antioxidant activity by ABTS free radical scavenging method

ABTS radicals were generated by mixing 7 mM ABTS (Sigma Aldrich Chemical Company, St Louis, MO, USA) with 4.95 mM potassium persulfate (1:1 *v/v*) and incubating in dark for 16 hours. The solution was diluted with ethanol to achieve an absorbance of 1–1.5 at 734 nm.

For each sample, 0.1 ml of ethanolic extract (100–500 mg/ml, in triplicate) was mixed with 3.9 ml of ABTS solution. Absorbance reduction was measured at 734 nm using a UV-30 spectrophotometer, with ethanol as the blank. All samples were assayed

Table 2.

Tests for quantitative analysis of phytochemicals

Phytochemicals	Tests
1. Flavonoids	Aluminium colorimetric method [30]
2. Alkaloids	[31, 32]
3. Phenols	Folin-Ciocalteu colorimetric method [33]
4. Tannins	Folin-Ciocalteu colorimetric method [34]
5. Terpenoids	[35]
6. Steroids	[36]
7. Cardiac glycosides	[36]
8. Saponins	[37]

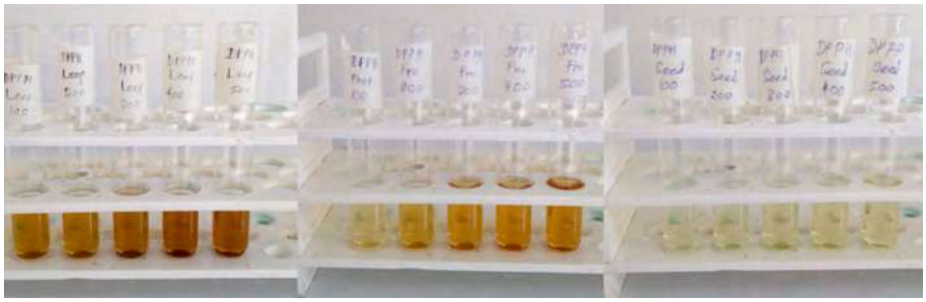


Figure 4.

Samples prepared for DPPH assay

in triplicate, and the average values were used for analysis (fig. 5).

ABTS scavenging ability (%) was calculated as:

$$\text{ABTS Scavenged (\%)} = \frac{A_B - A_A}{A_B} \times 100,$$

where $\mathbf{A_B}$ is the blank absorbance at 0 min and $\mathbf{A_A}$ is the antioxidant absorbance at 30 min.

The concentration of the extract required to scavenge 50% of the ABTS radicals (IC_{50}) was calculated by plotting a graph of % inhibition versus concentration.

Figure 5.

Samples prepared for ABTS assay

Determination of median inhibitory concentration (IC_{50})

The IC_{50} value, representing the concentration required to scavenge 50% of free radicals, was determined from the concentration-response curve for both DPPH and ABTS assays.

Evaluation of antimicrobial activity of extracts of *P. emblica* plant parts against selected bacterial strains and fungal cultures

Assay of antibacterial activity using agar well diffusion method

Antibacterial activity was assessed using the agar well diffusion method against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria provided by the Department of Botany, Faculty of Science, University of Jaffna, as mentioned in table 3. All test strains were preserved on nutrient agar (Avon Pharmo Chem (Pvt) Ltd) slants at 4°C and subcultured in nutrient agar (Avon Pharmo Chem (Pvt) Ltd) for 24 hours before experimentation. Following overnight incubation at 37°C, well-isolated colonies were aseptically picked using a sterile inoculating loop and transferred into 2 ml of sterile distilled water to prepare a bacterial suspension. The suspension was thoroughly mixed to ensure homogeneity. The inoculum was then adjusted to match a 0.5 McFarland turbidity standard, corresponding to an approximate cell density of 1.5×10^8 CFU/ml.

A 0.1 ml aliquot of this bacterial suspension was then introduced into 10 ml of sterile saline and evenly spread onto nutrient agar (NA) plates using a sterile spreader. Wells were created in the agar using a sterile cork borer (0.5 cm in diameter) into which 20 μl of each plant extract was dispensed. The plates were incubated at 37°C for 24 hours.

Sterile distilled water and streptomycin (100 μg/μl) (Avon Pharmo Chem (Pvt) Ltd.) served as negative and positive controls, respectively. After incubation, the inhibition zones around each well were measured (in millimetres) along four predetermined axes, and the average values were recorded. All experiments were performed in triplicate to ensure reliability.

Assay of antifungal activity by using in-vitro antifungal bio assay

The antifungal activity of plant extracts was assessed against three fungal strains (*Mucor* spp., *Aspergillus* spp., and *Fusarium* spp.) obtained from the Department of Botany, Faculty of Science, University of Jaffna, using the disc diffusion method (tab. 4). Prior to testing, each fungal strain was subcultured on Potato Dextrose Agar (PDA) (Avon Pharmo Chem (Pvt) Ltd).

Crude ethanolic, petroleum ether, and acetone extracts of plant materials were evaluated for antifungal effects. For inoculum preparation, a 9 mm mycelial plug from each fungal culture was transferred to PDA plates supplemented with streptomycin (100 μg/ml) and incubated in darkness at a room temperature with incubation duration adjusted based on fungal growth rates.

Sterile filter paper discs (14 mm in diameter) were saturated with 20 μl of each extract and placed at the centre of fresh PDA plates. A 9 mm fungal plug was then positioned directly onto each disc. The plates were incubated at a room temperature, and inhibition zone radii were measured after six days, once fungal growth reached its maximum extent [10].

The fungicide Homai (20 μl) and sterile distilled water (20 μl) served as positive and negative controls, respectively. All assays were conducted in triplicate. The percentage of fungal growth inhibition was calculated using the formula:

Table 3.

Bacterial strains used in the study

Name	Type	ATTC No.
Escherichia coli	Gram negative	25922
Staphylococcus aureus	Gram positive	29213
Pseudomonas aeruginosa	Gram negative	27853

Table 4.

Fungal strains used in the study

Name	Division
Mucor sp.	Mucoromycota
Aspergillus sp.	Ascomycota
Fusarium sp.	Ascomycota

$$\text{Inhibition of fungal growth (\%)} = \frac{D_0 - D}{D_0},$$

where $\mathbf{D_0}$ is the diameter of the negative control and **D** is the mean diameter of fungal growth in the presence of the extract.

Statistical analyses of the results

All experiments were done in triplicate. Data were analysed using one-way ANOVA in Minitab 17, with Tukey's multiple comparison test applied at a significance level of $p<0.05$. Results were presented as mean ± standard deviation (SD) [11].

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals

The preliminary phytochemical screening of leaves, fruits and seeds of *P. emblica* were performed for the detection of phytochemicals. The chemical composition of *P. emblica* extracts was evaluated through qualitative phytochemical screening, as shown in table 5.

According to the study, vitamin C, tannins, flavonoids, phenols, alkaloids, glycosides, phytosterols, quinones, anthraquinones, coumarins, carboxylic acids, reducing sugars, carbohydrates, xanthoproteins, proteins and amino acids were found in leaves, fruits and seeds of *P. emblica*. The results indicated that all three parts of *P. emblica* are rich in flavonoids, alkaloids, tannins, phenols, vitamin C, carboxylic acids and reducing sugars. Terpenoids and saponins were present in all extracts of leaf and seed but absent in fruit extracts. Steroids were present in all seed extracts and in aqueous extract of leaf but absent in all extracts of fruit and in ethanolic and petroleum ether extracts of leaf. Glycosides were present in all extracts of leaf, all extracts of seed and in ethanolic extract of fruit but absent in petroleum ether and aqueous extracts of fruit. Carotenoids and resins were present in all extracts of fruit and in ethanolic extract of seed whereas absent in all the leaf extracts, petroleum ether and aqueous extracts of seed. Diterpenes were present only in petroleum ether extract of leaf. Fixed oils and fats were present in the petroleum ether extracts of leaf, fruit and seed and in ethanolic extract of seed. Lignins, phlobatannins and anthocyanins were absent in all extracts of leaf, fruit and seed.

It was also observed that ethanolic extraction yielded the highest number of phytochemicals compared to aqueous and petroleum ether extracts. The phytochemical screening of the ethanolic, petroleum ether and aqueous extracts of *P. emblica* plant parts indicate the presence of phytochemicals in the order of ethanolic > aqueous > petroleum ether extracts.

P. emblica has been extensively documented in Ayurvedic medicine for its therapeutic applications, serving as a key ingredient in numerous traditional and modern pharmaceutical formulations.

All parts of the *P. emblica* plant are utilized for medicinal purposes, with the fruit being particularly valued. In Ayurveda, it is recognized as a powerful *Rasayana* (rejuvenator), and in traditional medicine, it has been used to treat conditions such as diarrhoea, jaundice, and inflammation. Different parts of the plant exhibit a range of therapeutic properties, including antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcerogenic, hepatoprotective, gastroprotective, and chemopreventive effects [12].

Phytochemical analysis of the ethanolic fruit extract of *P. emblica* L. revealed the presence of flavonoids, alkaloids, tannins, steroids, reducing sugars, and gums [13]. *P. emblica* is known to contain a wide range of phytochemicals, including fixed oils, phosphatides, essential oils, tannins, minerals, vitamins, amino acids, fatty acids, and glycosides. It has been extensively studied for its diverse pharmaceutical properties, such as antimicrobial,

Table 5.

Results of qualitative phytochemical screening of *Phyllanthus emblica* leaves, fruits and seeds

Phytochemical	Leaves			Fruits			Seeds		
	Eth	PE	Aq	Eth	PE	Aq	Eth	PE	Aq
Flavonoids	+++	++	–	++	+	–	+	+	–
Phenols	++	–	++	++	–	++	++	–	++
Tannins	++	–	++	++	–	++	++	–	++
Alkaloids	+	+	–	++	+	++	+	+	–
Terpenoids	+	+	+	–	–	–	++	+	++
Saponins	++	+	++	–	–	–	+	+	+
Glycosides	++	++	++	+	–	–	+	+	+
Anthocyanins	–	–	–	–	–	–	–	–	–
Steroids	–	–	+	–	–	–	++	+	++
Phytosterols	++	++	+	+	+	–	+	+	+
Anthraquinones	+	+	+	++	++	++	+	+	+
Quinones	+	+	+	++	++	++	+	–	+
Coumarins	++	+	++	+	–	+	+	–	+
Carboxylic acids	++	++	++	+++	+++	+++	++	++	++
Reducing sugar	+	+	+	+++	+++	+++	+	+	+
Xanthoproteins	+	–	+	+	–	–	+	–	+
Proteins and amino acids	+	+	+	+	+	–	+	+	+
Vitamin C	+	+	+	+++	+++	+++	+	+	–
Lignins	–	–	–	–	–	–	–	–	–
Carotenoids	–	–	–	++	++	++	+	–	–

Et – ethanolic extract; PE – petroleum ether extract; Aq – aqueous extract; +++ – abundant; ++ – average; + – present – – absent

antioxidant, anti-inflammatory, analgesic, antipyretic, adaptogenic, hepatoprotective, antitumour, and antiulcerogenic effects. These therapeutic activities have been observed in both combined formulations and when using *P. emblica* alone [2].

P. emblica is exceedingly healthful and serves as a valuable dietary source of vitamin C, amino acids, and essential minerals. It also comprehends a wide variety of bioactive compounds, including phenolic compounds, tannins, phyllembelic acid, phyllembelin, rutin, curcuminoids, and emblicol [12].

Phytochemical analysis of the dried fruits of *P. emblica* (Amla) revealed the presence of phenols, flavonoids, non-flavonoids, tannins, alkaloids, saponins, and phytosterols across various extracts, including diethyl ether, ethyl acetate, butanol, and aqueous extracts [14].

Phytochemical analyses of *P. emblica* plant parts have identified significant constituents which contribute to its broad-spectrum biological activities.

Quantitative analysis of phytochemicals

The quantitative analysis (tab. 6) revealed that ethanolic extracts contained the highest concentrations of bioactive compounds across all plant parts studied. Among these, the leaf extracts were particularly rich in flavonoids (32.24±1.87 μg QE/g) and tannins (505.25±1.67 μg TAE/g), while fruit extracts exhibited a notable abundance of phenolic compounds (24.82±1.72 μg GAE/g). The highest alkaloid content was observed in the fruit powder (56.88±2.88 mg/g).

The ethanolic extract of leaves had the highest flavonoid concentration (32.24±1.87 μg/ml), while the petroleum ether extract of fruits had the lowest (2.51±0.29 μg/ml). In general, leaves and fruits contained higher flavonoid levels than seeds. The total flavonoid content was calculated based on the aluminium chloride colorimetric method and quantified using a quercetin standard curve

Table 6.

Results of quantitative estimation of selected phytochemicals of *Phyllanthus emblica*

Phytochemical content	Leaf	Fruit	Seed
Flavonoid content [μg QE/g]	32.24±1.87 (Et)	25.07±1.77 (Et)	9.68±0.08 (Et)
Phenol content [μg GAE/g]	24.46±0.48 (Et)	24.82±1.72 (Aq)	17.27±0.71 (Et)
Alkaloid content [mg/g]	27.73±1.48	56.88±2.88	14.37±0.25
Tannin content [μg TAE/g]	505.25±1.67 (Et)	339.47±1.88 (Aq)	247.21±2.83 (Et)
Cardiac glycoside content [mg/g]	24.85±0.62 (PE)	20.75±0.67 (PE)	23.51±0.58 (PE)
Terpenoid content [mg/g]	0.026±0.002	0.032±0.007	0.444±0.113
Saponin content [mg/g]	0.24±0.03	0	0.08±0.03
Steroid content [μg DE/g]	1.89±0.54 (Et)	1.72±0.15 (PE)	1.92±0.24 (Et)

Et – ethanolic extract; PE – petroleum ether extract; Aq – aqueous extract

(y = 0.0283x). The results were reported as quercetin equivalents (QE/g). According to Fitriansyah *et al.* (2018), the ethanolic leaf extract of *P. emblica* had the highest flavonoid content, which supports this [15].

Phenolic content peaked in the aqueous extract of fruits (24.83±1.73 μg/ml), with the lowest value recorded in the petroleum ether extract of fruits (0.21±0.08 μg/ml). Similar to flavonoids, phenolic content was generally higher in leaves and fruits compared to seeds. Previous studies have identified phenolic compounds – particularly hydrolysable tannins (both ellagitannins and gallotannins), catechins, and terpenoids – as the principal bioactive constituents of *P. emblica* [16].

Tannin content was highest in the ethanolic leaf extract (505.26±1.67 μg/ml) and lowest in the petroleum ether fruit extract (73.35 ± 2.03 μg/ml). As with other compounds, leaves and fruits generally showed higher tannin levels than seeds. It has been shown in several *in-vitro* and *in-vivo* studies that hydrolysable tannins, including putranjivain A, galloyl-HHDP-glucose, elaeocarpusin, mucic acid gallate, mucic acid lactone gallate, monogalloyl glucose, gallic acid, digalloylglucose, and chebulagicacid, those have antidiabetic, antimicrobial, anti-inflammatory, and immunomodulatory effects [17].

Steroid content was highest in the aqueous fruit extract (2.374±0.425 μg/ml) and lowest in the petroleum ether leaf extract (1.132±0.307 μg/ml). Fruits and seeds exhibited higher steroid levels compared to leaves. A range of phytosterol oxidation products was isolated from *Phyllanthus emblica*, including two new steroids – 5α,6β,7α-trihydroxysitosterol and 7α-acetoxysitosterol – along with twelve known steroids [18]. *P. emblica* has been reported to contain β-sitosterol-3-O-β-D-glucoside. Sterols are known to lower blood plasma cholesterol levels by interfering with cholesterol esterification. Reports suggest that sterols bind to intestinal mucosal cells and disrupt the transport of cholesteryl esters by interacting with lipoproteins, thereby reducing their movement into the bloodstream [19].

The highest cardiac glycoside content was observed in the petroleum ether extract of leaves (24.850±0.621 mg/g), while the ethanolic extract of seeds recorded the lowest level (14.700±0.604 mg/g). Across all plant parts and extract types, cardiac glycoside content ranged between 14–25 mg/g, with petroleum ether extracts consistently showing the highest levels.

Terpenoid content reached its peak in seeds (0.444±0.113 mg/g) and was lowest in leaves (0.027±0.002 mg/g), with seeds showing significantly higher levels than the other plant parts. According to Kumar *et al.* (2017), *P. emblica* is a source of terpenoids including betulinic acid, ursolic acid and oleanolic acid which exhibit a broad range of pharmacological activities such as anti-inflammatory, anti-cancer, antioxidant, and hepatoprotective effects [20].

Saponin content was highest in leaves (0.237±0.031 mg/g) and absent in fruits (0 mg/g). Generally, saponin levels were higher in leaves and seeds than in fruits. According to a phytochemical

screening conducted by Dhale and Mogle, saponins were detected in both leaf and fruit extracts of *P. emblica* prepared using various solvents [21].

Alkaloid content was greatest in fruits (56.88± 2.88 mg/g) and lowest in seeds (14.37±0.25 mg/g), with fruits consistently exhibiting higher levels than leaves and seeds. According to Ahmad *et al.* (2021), chromatographic and IR spectral analysis confirmed the presence of alkaloids such as phyllantidine and phyllantine [4]. Phyllantidine, an alkaloid present in the fruits of *P. emblica*, has demonstrated neuropharmacological activity [22].

Evaluation of antioxidant activity of leaves, fruits and seeds of *P. emblica* using DPPH assay and ABTS assay

DPPH and ABTS free radical scavenging assays revealed that ethanolic fruit extracts demonstrated the strongest antioxidant activity, with IC_{50} values of 130.649±0.918 mg/ml (DPPH) and 266.178±0.777 mg/ml (ABTS). Table 7 and Table 8 indicate the IC_{50} values obtained for all DPPH and ABTS assays. Figure 6 indicates DPPH free radial scavenging activity of *P. emblica* plant parts in different concentrations. Figure 7 indicates ABTS free radial scavenging activity of *P. emblica* plant parts in different concentrations.

The evaluation of DPPH activity of *P. emblica* focuses on its antioxidant properties, which are primarily due to its rich content of bioactive compounds like phenolics, flavonoids, and vitamin C. The DPPH assay is widely used to assess the ability of extracts to scavenge free radicals, reflecting their potential to combat oxidative stress.

Research highlights that methanolic and ethanolic extracts of *P. emblica* exhibit significant DPPH scavenging activity, correlating with their high phenolic content.

In studies on *P. emblica*, its extracts have shown significant ABTS radical scavenging activity, attributed to its high content of phenolic compounds, flavonoids, and vitamin C. With a strong antioxidant

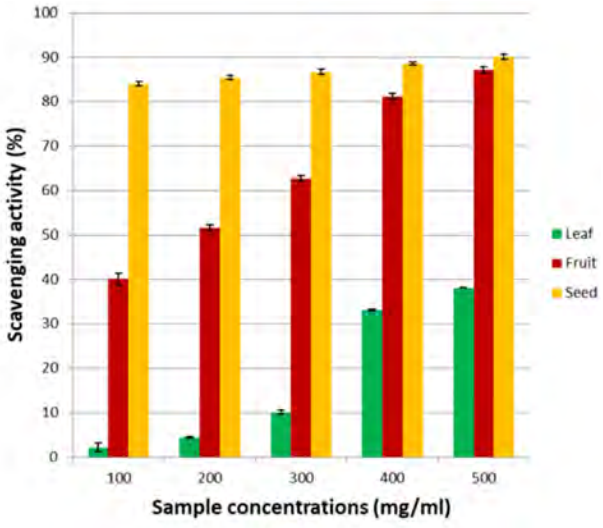


Figure 6.

DPPH radical scavenging activity of *P. emblica* plant parts in different concentrations

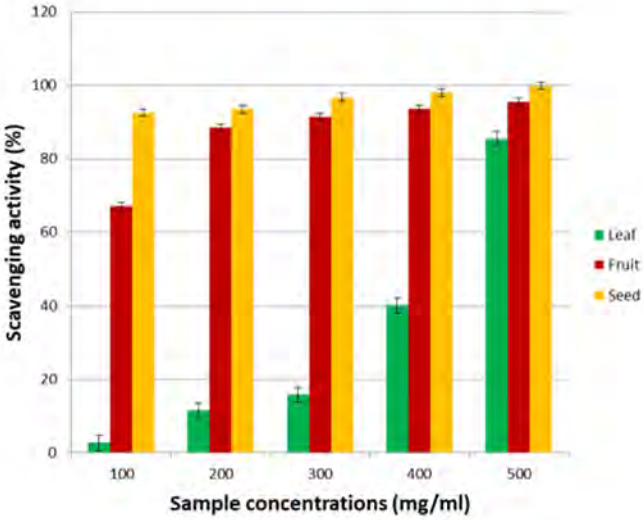


Figure 7.

ABTS radical scavenging activity of *P. emblica* plant parts in different concentrations

Table 7.

Antioxidant activity based on IC_{50} values of DPPH assay

Sample	DPPH [IC_{50} mg/ml]
Leaf	65.712±0.615
Fruit	417.14±0.82
Seed	2115.22±0.92

Table 8 .

Antioxidant activity based on IC_{50} values of ABTS assay

Sample	ABTS [IC_{50} mg/ml]
Leaf	203.206±0.406
Fruit	896.308±0.880
Seed	2725.789±0.777

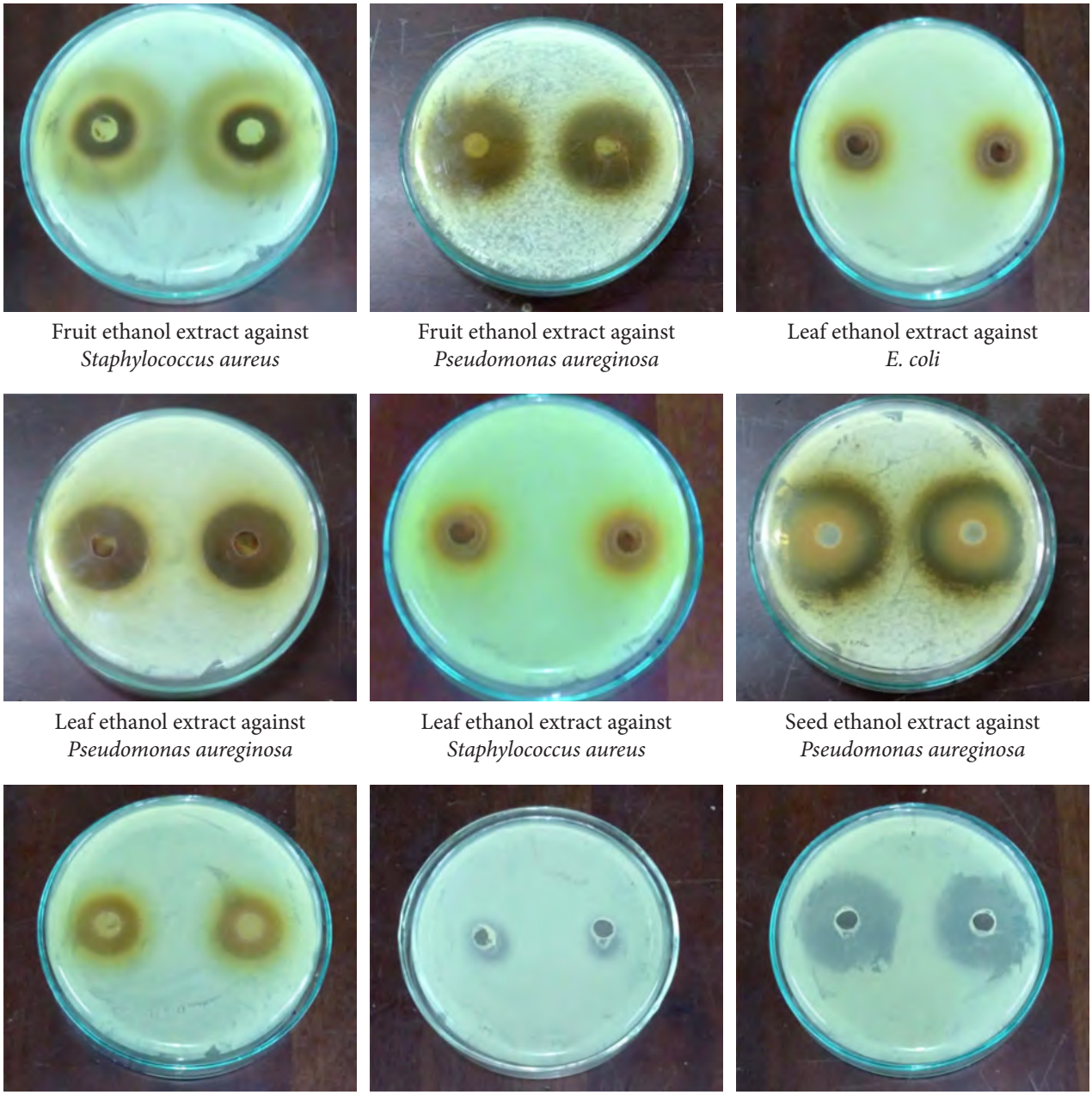
capacity, these bioactive substances efficiently donate electrons or hydrogen atoms to counteract free radicals. Recent studies have demonstrated that this characteristic is associated with a number of health advantages, such as anti-inflammatory, neuroprotective, and anti-aging properties [23].

Evaluation of antimicrobial activity of extracts of leaves, fruits and seeds of *P. emblica* against selected bacterial strains and fungal cultures

Ethanolic fruit extracts exhibited the strongest antibacterial effects, with inhibition zones of 39.67±0.58 mm against *S. aureus*, 27.00±0.05 mm against *E. coli*, and 35.33±0.57 mm against *P. aeruginosa* (fig. 8).

The variation in mean diameter of inhibition zones of different bacterial strains treated with extracts of *P. emblica* is illustrated in figure 9.

The antibacterial efficacy of plant extracts against *Pseudomonas aeruginosa*, *Staphylococcus aureus*,



Fruit ethanol extract against *Staphylococcus aureus*

Fruit ethanol extract against *Pseudomonas aureginosa*

Leaf ethanol extract against *E. coli*

Leaf ethanol extract against *Pseudomonas aureginosa*

Leaf ethanol extract against *Staphylococcus aureus*

Seed ethanol extract against *Pseudomonas aureginosa*

Fruit ethanol extract against *E. coli*

Distilled water against *E. coli* (Negative control)

Streptomycin against *E. coli* (Positive control)

Figure 8.

Observations for evaluation of antibacterial activity of *P. emblica* extracts

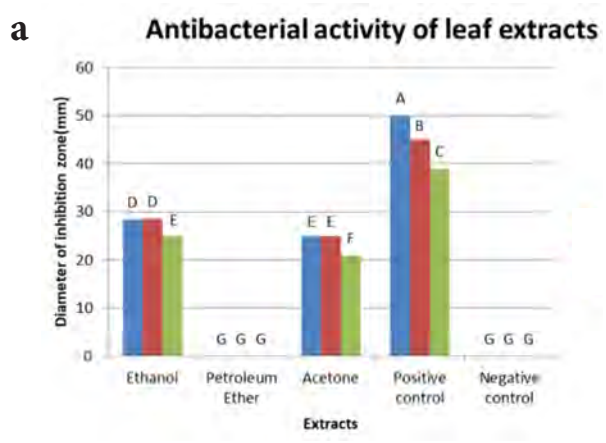


Figure 9a.

Variation in mean diameter of inhibition zones of different bacterial strains by extracts of *Phyllanthus emblica* L. leaves

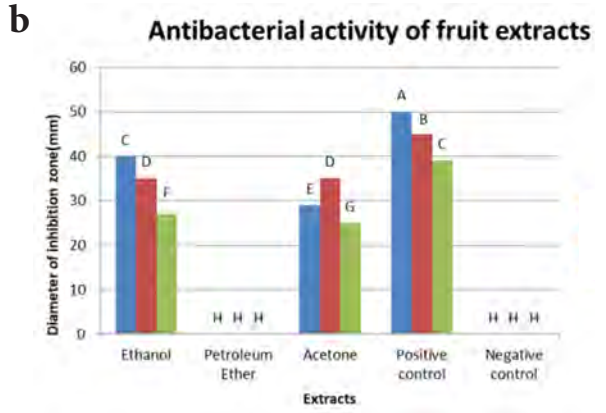


Figure 9b.

Variation in mean diameter of inhibition zones of different bacterial strains by extracts of *Phyllanthus emblica* L. fruits

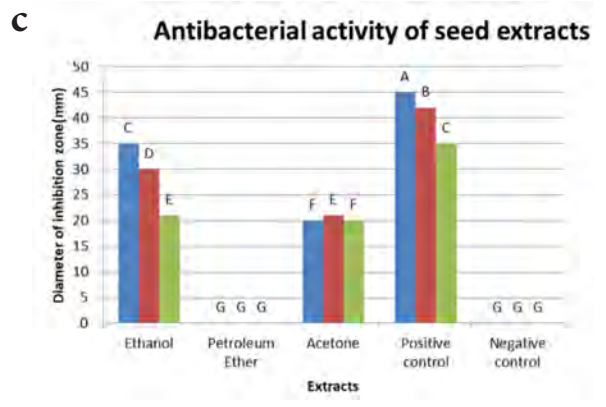


Figure 9c.

Variation in mean diameter of inhibition zones of different bacterial strains by extracts of *Phyllanthus emblica* L. seeds.

Staphylococcus *Pseudomonas* *E.coli*

and *Escherichia coli* varied because of variations in bacterial cell wall structure and the ways in which bioactive chemicals act. *E. coli* and *P. aeruginosa* are gram-negative bacteria which are often less susceptible to plant extracts because of their outer membrane, which acts as a barrier against many compounds. *S. aureus*, a gram-positive bacterium, is in general more sensitive to plant extracts due to its thick peptidoglycan layer, which lacks the protective outer membrane of gram-negative bacteria.

Antifungal activity was the highest against *Fusarium* spp., with growth inhibition percentage of 56.49% in ethanolic leaf extracts.

All three types of leaf extracts displayed moderate antifungal activity, with notable inhibition against *Aspergillus* and *Fusarium* species. However, *Mucor* spp. showed the least susceptibility, indicating a higher level of resistance to the extracts.

Similarly, moderate antifungal activity was observed in fruit and seed extracts. Among the tested fungi, *Fusarium* spp. showed the highest growth inhibition, followed by *Aspergillus* spp., while *Mucor* spp. again proved to be the most resistant (fig. 10).

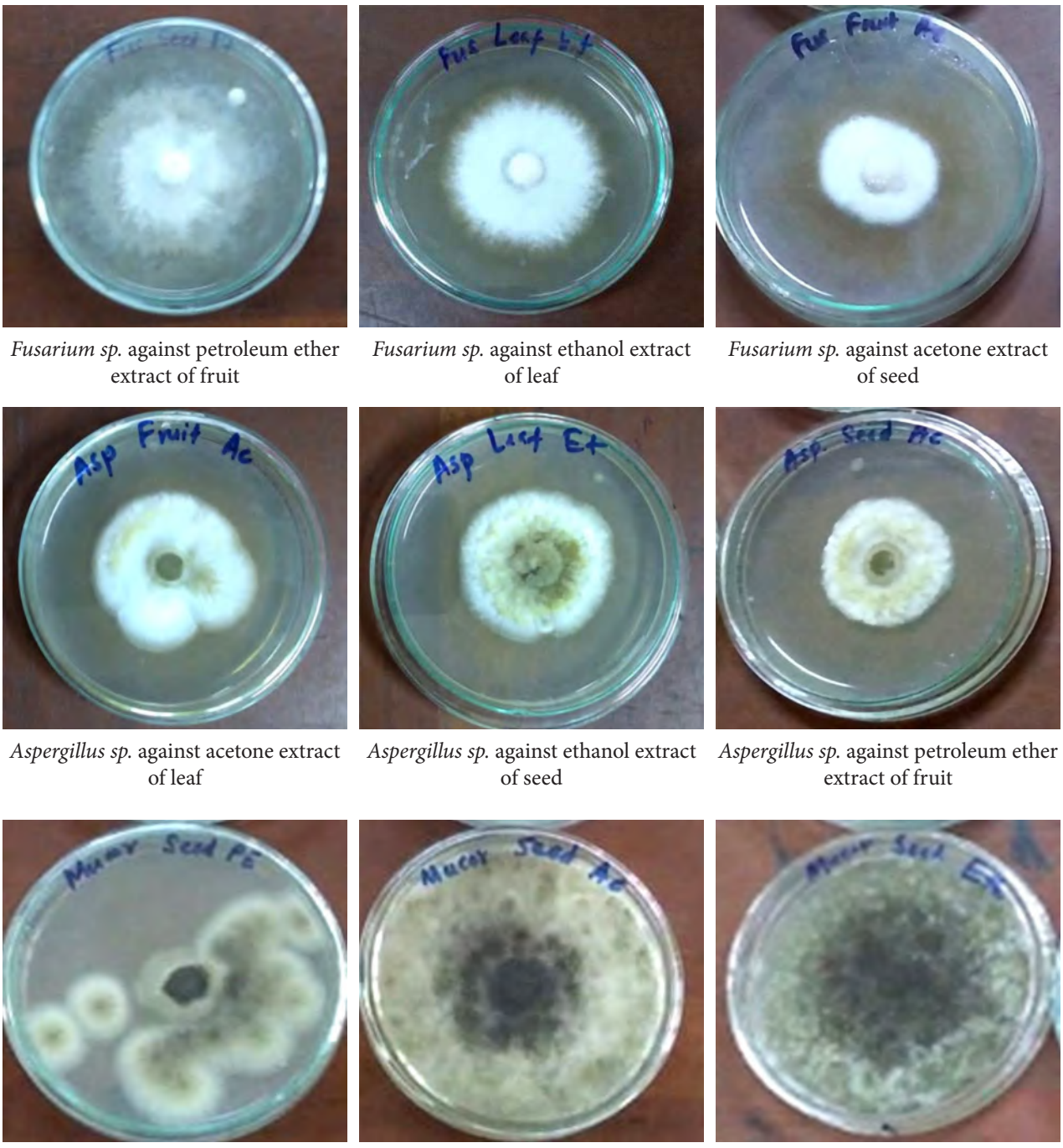
Figure 11a, 11b, and 11c shows the positive control for *Fusarium* spp., *Aspergillus* spp., and *Mucor* spp. respectively, where Homai fungicide was used to assess the maximum expected anti-fungal activity. In contrast, Figure 12a, 12b, and 12c depicts the negative control for *Fusarium* spp., *Aspergillus* spp., and *Mucor* spp. respectively, using sterile distilled water to confirm the absence of antifungal effects without treatment.

The variation in the percentage of inhibition of fungal growth among different fungal strains treated with extracts of *P. emblica* is illustrated in figure 13.

These findings suggest that *P. emblica* extracts, particularly ethanolic ones, possess broad-spectrum antimicrobial properties, though their efficacy varies depending on the microorganism.

CONCLUSION

The qualitative phytochemical analysis indicated the presence of phytochemicals like vitamin C, tannins, flavonoids, phenols, alkaloids, coumarins, cardiac glycosides, terpenoids, saponins, steroids and phytosterols in all three tested plant parts: leaves, fruits and seeds of *P. emblica.* Phytochemicals such as anthocyanins, phlobatannins and lignins were not present in any of these plant parts. Ethanolic extracts of the samples showed highest amount of phytochemicals.



Fusarium sp. against petroleum ether extract of fruit | *Fusarium sp.* against ethanol extract of leaf | *Fusarium sp.* against acetone extract of seed

Aspergillus sp. against acetone extract of leaf | *Aspergillus sp.* against ethanol extract of seed | *Aspergillus sp.* against petroleum ether extract of fruit

Mucor sp. against petroleum ether extract of seed | *Mucor sp.* against ethanol extract of leaf | *Mucor sp.* against acetone extract of fruit

Figure 10.

Observations of evaluation of antifungal activity of *P. emblica* extracts

The quantitative phytochemical analysis indicated that the leaves have the highest concentration of flavonoids, tannins, cardiac glycosides, and saponins while fruits have the highest concentration of vitamin C, phenols and alkaloids and seeds have the highest concentration of steroids and terpenoids.

According to the findings of the analysis of the antioxidant activity of ethanolic extracts of *P. emblica* leaves, fruits, and seeds, fruit extracts exhibited the highest antioxidant activity, while seed extracts displayed the lowest. Fruits showed the strongest antioxidant activity due to their high levels

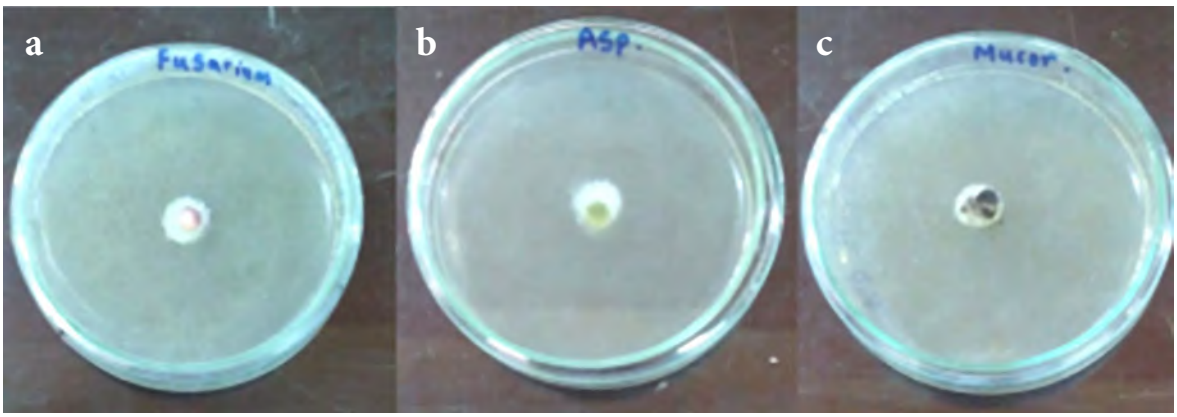


Figure 11 a, b, c.

Positive control of *Fusarium* sp., *Aspergillus* sp. and *Mucor* sp. respectively.
Homai fungicide is used as a positive control

of flavonoids, polyphenols, vitamin C, and tannins. Hence, it can be concluded that highest antioxidant activity is shown by the plant part that is rich in phytochemicals. Primary phytochemical responsible for antioxidant activity of *P. emblica* is ascorbic acid (vitamin C), however other compounds like flavonoids, tannins and polyphenols also contribute to antioxidant activity.

The findings from the assessment of the antibacterial properties of *P. emblica* leaves, fruits, and seeds in ethanolic, petroleum ether, and acetone extracts against a particular bacterial strain (*Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*) showed that all three plant parts exhibited antibacterial activity against these bacterial samples. Extracts of the fruit showed the highest antibacterial activity among the three plant parts. Antibacterial activity was observed in the order: fruit extracts > seed extracts > leaf extracts.

The findings of the ethanolic, petroleum ether, and acetone extracts of leaves, fruits, and seeds of *P. emblica* against specific fungal cultures (*Mucor* spp., *Aspergillus* spp., and *Fusarium* spp.) demonstrated antifungal activity against tested fungal samples. Antifungal activity was observed in the order: leaf extracts > seed extracts > fruit extracts.

This study proves *P. emblica* as a potent natural source of antioxidants and antimicrobials. Its rich phytochemical composition supports traditional medicinal applications and highlights its potential for pharmaceutical development targeting oxidative stress-related disorders and microbial infections.

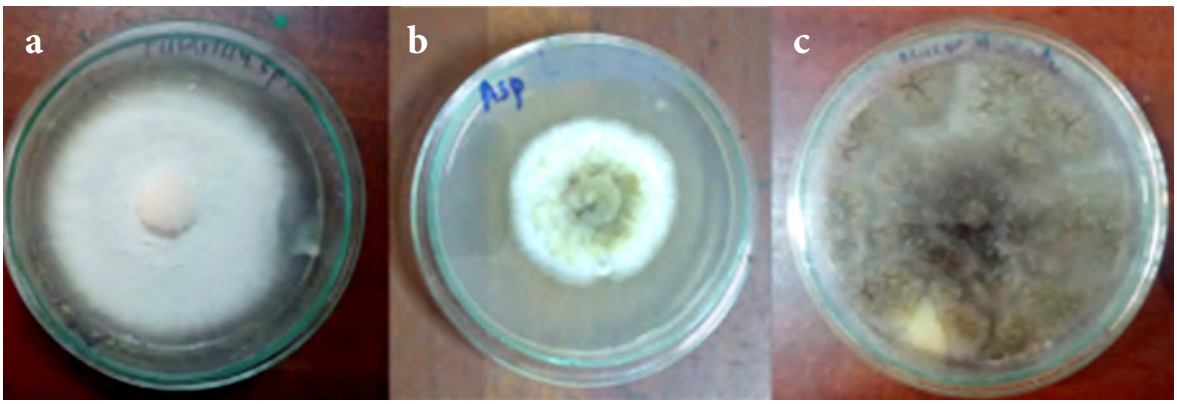


Figure 12 a, b, c.

Negative control of *Fusarium* sp., *Aspergillus* sp. and *Mucor* sp. respectively.
Sterile distilled water is used as negative control

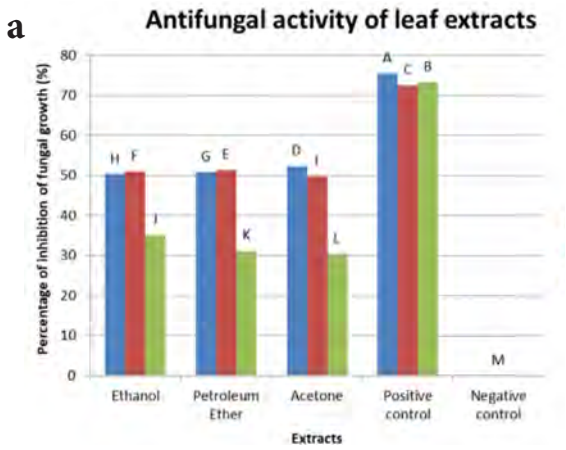


Figure 13a.

Variation in percentage of inhibition of fungal growth of different fungal strains by extracts of *Phyllanthus emblica* L. leaves

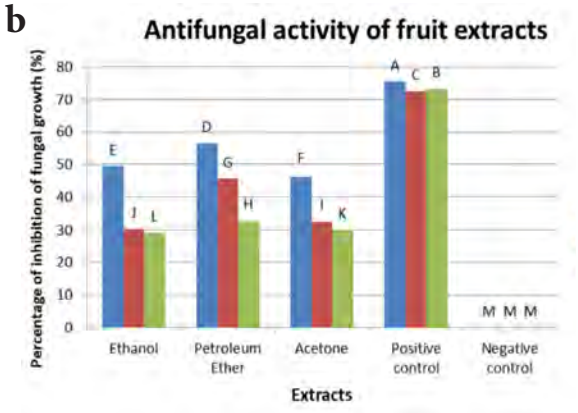


Figure 13b.

Variation in percentage of inhibition of fungal growth of different fungal strains by extracts of *Phyllanthus emblica* L. fruits

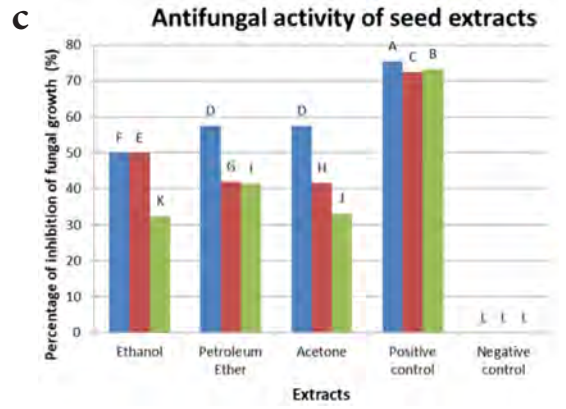


Figure 13c.

Variation in percentage of inhibition of fungal growth of different fungal strains by extracts of *Phyllanthus emblica* L. seeds

Fusarium *Aspergillus* *Mucor*

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Ethical approval

The research conducted is not related to either human or animal use.

Conflict of interest

Authors declare no conflict of interest.

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