

## Phytochemical Analysis, Antioxidant and Antibacterial Properties of *Phyllanthus emblica* Leaf Extracts against Selected Bacterial Isolates

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### ABSTRACT

**Background:** A major challenge in global health care is the need for novel, effective and affordable medicines to treat microbial infections, as up to one-half of deaths are due to infectious diseases. The main concern is the development of antimicrobial-resistant bacteria species from a number of factors which include the prevalent and sometimes inappropriate use of antibiotics. Against this backdrop, the discovery of alternative drug classes to treat such infectious diseases is urgently required. Plants have an amazing ability to produce a wide variety of secondary metabolites which are the source of plant-derived antimicrobial substances.

**Methods:** Present study is carried out to investigate *in vitro* antibacterial activity of *Phyllanthus emblica* leaf extracts against the selected bacterial strains *Escherichia coli* and *Staphylococcus aureus*. Further, antioxidant activity and phytochemical analysis of the effective solvent extracts were done. Agar well diffusion was used for testing antibacterial activity. DPPH assay was carried out to evaluate antioxidant activity and phytochemical screening was tested by standard procedure.

**Results:** Results revealed that maximum antibacterial activity was displayed by ethanol extract of *P. emblica* against both the test microorganisms. Acetone extract of *P. emblica* embarked significant radical scavenging potential. And therefore, ethanol and acetone extract of *P. emblica* when analysed for the presence of phytochemicals revealed a number of phytocompounds.

**Conclusion:** It may be concluded that as plant extracts showed great antibacterial potential against microorganisms and revealed a rich source of antioxidant properties, they can be

used as a cure for infectious diseases caused by resistant microbes.

**Keywords:** Antibacterial activity; *Phyllanthus emblica*; Plant extracts; Agar well diffusion; Antioxidant activity; DPPH Assay; Phytochemical analysis

### 1. INTRODUCTION

Antibiotics are undeniably one of the most important therapeutic discoveries of the 20<sup>th</sup> century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from the synthetic products. [1] With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs. [2] This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics. [3,4]

Pharmacological industries have shaped several new antibiotics and in the last three decades resistance to these drugs by microorganisms has increased. In general, bacteria are the microorganisms which have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. [5] Antibiotic resistance has increased substantially in the recent years and is posing an ever-increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance

inhibitors from plants. [6,7]

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect the plant from various diseases. Many plants species are reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes which is therefore, should be utilized to combat the disease-causing pathogens. [8-10] Medicinal plants are being used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs. [11-13] The aim of this study was to screen the *in vitro* antibacterial activity of *Phyllanthus emblica* leaf extracts against the selected bacterial strains *Escherichia coli* and *Staphylococcus aureus*, and to evaluate the antioxidant potential and secondary metabolites in the effective solvent extracts.

*Phyllanthus emblica*, is known as Indian gooseberry or Amla, is coming under family *Euphorbiaceae*, is a main herbal drug utilized in unani and ayurvedic systems of medicine. It is used as a medicine and as a tonic to build up lost energy and vitality. *P. emblica* is extremely nutritious and can be a chief dietary source of vitamin C, amino acids, and minerals. The fruit and leaves are used either alone or in combination with other plants to treat, many ailments such as common cold and fever; as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-inflammatory, antipyretic, hair tonic, and as a digestive. Additionally, plant parts show antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcerogenic, hepatoprotective, gastroprotective, and chemo preventive properties. [14]

## 2. MATERIALS AND METHODS

### Collections of test materials

Leaves of *Phyllanthus emblica* were collected from the Koduvayur locale of Palakkad district, Kerala, and the specimens were identified, certified and the voucher specimen number (BSI/SRC/5/23/2018/Tech/2478) were deposited at the Botanical Survey of India, Southern Circle, Coimbatore.

### Preparation of leaf powder and extracts

Fresh leaves of *P. emblica* were air dried under shade. Dried leaves were powdered using an electric pulverizer. Fine powder was obtained by sieving. The powder was subjected to extraction. [15,16] Acetone extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity. The leaf extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C. The residue obtained was stored in tightly closed glass vials in the refrigerator for further use. Antibacterial activity was investigated.

### Test microorganism

The bacterial strains used were the clinical isolates obtained from a Hospital in Coimbatore. The bacterial strains used were *E. coli* and *S. aureus*.

### 2.1 Antibacterial assay

The activity of various solvent extracts of leaves of *P. emblica* on selected bacterial strains was assayed by agar well diffusion method. For agar well diffusion, method of Murray *et al* [17] later modified by Olurinola [18] was used. Antibacterial susceptibility was tested on solid media in petriplates. For bacteria Nutrient agar was used for developing surface colony growth.

### Reagents: Nutrient Agar

Nutrient agar medium was prepared and poured on to the petriplates and was left on sterile surface until the agar has solidified. The plates were swabbed (sterile cotton swabs) with 24 h old culture of bacterial strains. Wells were made in each of these plates using sterile cork borer. Stock solution of each solvent extract viz.,

Acetone, Chloroform and Ethanol was prepared at a concentration of 1 mg/ml. About 50µl of different solvent extracts of the leaves of *P. emblica* was added using sterile syringe into the wells and allowed to diffuse at room temperature for 2 h. Amoxicillin was used as positive antibacterial control.

The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around well. [19] Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

### Statistical Analysis

The antimicrobial data was interpreted by calculating standard deviation and mean of three replicates.

### 2.2 Antioxidant assay

#### DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang *et al.* [20] The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 518 nm.

#### Principle

2,2-diphenyl-1-picrylhydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity.

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

#### Reagent preparation

0.1mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared by dissolving 4 mg of DPPH in 100ml of methanol.

#### Procedure

To 0.01 ml to 0.05 ml of the fraction, 0.5 ml of a methanolic solution of DPPH and 0.49 ml of methanol were added. The mixture was allowed to react at room temperature for 30 minutes in the dark. Methanol served as a blank and DPPH in methanol, without plant extract fraction, served as the positive control. After 30 minutes of incubation, the decolorization of the purple to yellow colour was measured at 518 nm. The radical scavenging activity was calculated as,

$$\% \text{ RSA} = \frac{\{(\text{Abs control} - \text{Abs sample})\}}{\text{Abs control}} \times 100$$

Where, *RSA* is the Radical Scavenging Activity; *Abs control* is the absorbance of DPPH radical + methanol; *Abs sample* is the absorbance of DPPH radical + plant extract.

### 2.3 Phytochemical screening

Preliminary phytochemical screening of highly effective *P. emblica* leaf extract was carried out using the standard procedures.

#### Test for Alkaloids

- **Mayer's test:** [21] 1 ml of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test:** [22] 1 ml of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.
- **Hager's test:** [23] 1 ml of extract was treated with 1 or 2 ml of Hager's reagent and observed for the formation of prominent yellow precipitate.

#### Test for Tannins

- **Ferric chloride test:** [24] 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate.

#### Test for Phenols

- **Ferric chloride test:** [25] The extract (50 mg) was dissolved in 5 ml of distilled water and treated with few drops of 5%

ferric chloride and observed for the formation of dark green colour

- **Lead acetate test:** [26,27] The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

#### Test for Flavonoids

- **NaOH test:** [24] 1 ml the extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.
- **Lead acetate test:** [26,27] Fifty milligram of the extract was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

#### Test for Sterols

- **Liebermann-Burchard test:** [28] The extract (50 mg) was dissolved in 2 ml of acetic anhydride. To this one or two drop of Conc. H<sub>2</sub>SO<sub>4</sub> was added along the side of the test tube and observed for any colour change.

#### Test for Terpenoids

- **Liebermann-Burchard test:** [29] A little of extract (50 mg) was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of Conc. H<sub>2</sub>SO<sub>4</sub>. Change of colour from pink to violet indicates the presence of terpenoids.

#### Test for Saponins

- **Foam Test:** The extract (50 mg) or dry powder was diluted with distilled water and made up to 20 ml. The solution is vigorously shaken for 15 minutes and observed for the formation of 2 cm layer thick foam.

#### Test for Anthraquinones

- **Borntrager's test:** [30] Extract (0.2 g) to be tested was shaken with 10 ml of benzene and then filtered. Five ml of the 10% ammonia solution was added to the filtrate, shaken and observed for the

appearance of a pink, red or violet colour.

#### Test for Proteins

- **Ninhydrin test:** [31] Three drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of extract and observed for the present of characteristic purple colour.
- **Biuret test:** [31] Two ml of extract was treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added followed by excess of potassium hydroxide pellets and observed for the formation of pink ethanolic layer.

#### Test for Quinones

- **H<sub>2</sub>SO<sub>4</sub> test:** [27] To 1 ml of extract, 1 ml of Conc. H<sub>2</sub>SO<sub>4</sub> was added and observed for the formation of red colour.
- **HCl test:** [32,33] To 1 ml of the extract, 5 ml of HCl was added and observed for the presence of yellow colour precipitate.

### 3. RESULTS AND DISCUSSION

#### 3.1 Antibacterial activity of *P. emblica*

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. [34]

In the present study antibacterial activity of leaf extract of *P. emblica* are determined against different bacterial strains. Antibacterial activity was recorded as inhibition zone diameter measured in millimeter (mm).

#### Leaf extract of *P. emblica* against *E. coli*

Result of the antibacterial activity of *P. emblica* leaf extracts against *E. coli* is presented in Table 1 & Fig 1. In this study, against the bacterium *E. coli* maximum antibacterial activity was exhibited by ethanol extract in which zone of inhibition was reported as 29.2±2.3mm at a concentration of 40µl and 27.6±2.1mm at a concentration of 30µl respectively. Its positive control amoxicillin showed an inhibition zone of 32.1±2.6 mm. Duhri *et al* [35] has reported that the ethanol extract of *Origanum elongatum* leaves was the most effective against *E. coli* with 30.33±2.51

mm of diameter of inhibition zone. The ethanol extracts of *Myrtus communis*, *Thymbra capitata* leaves and *Punica*

*granatum* seeds also had significant antibacterial activity against *E. coli*.

**Table 1: Antibacterial activity of *P. emblica* leaf extracts against *E. coli* and *S. aureus***

Test bacteria	Treatments (µl)	Zone of inhibition (mm)		
		Acetone	Chloroform	Ethanol
<i>E. coli</i>	Positive cont	32.1±2.6	32.1±2.6	32.1±2.6
	10	19.8±1.0	9.0±0.5	22.6±1.4
	20	24.6±1.5	11.2±0.8	27±1.8
	30	26.2±1.9	11.4±0.8	27.6±2.1
	40	26.4±2.0	12.4±0.9	29.2±2.3
<i>S. aureus</i>	Positive cont	35.4±2.1	35.4±2.1	35.4±2.1
	10	16±0.5	8±0.2	24±0.8
	20	20.6±0.7	10.6±0.2	28.8±1.1
	30	23.2±0.8	11±0.3	30.2±1.3
	40	25.8±1.0	11.8±0.3	33.4±1.7

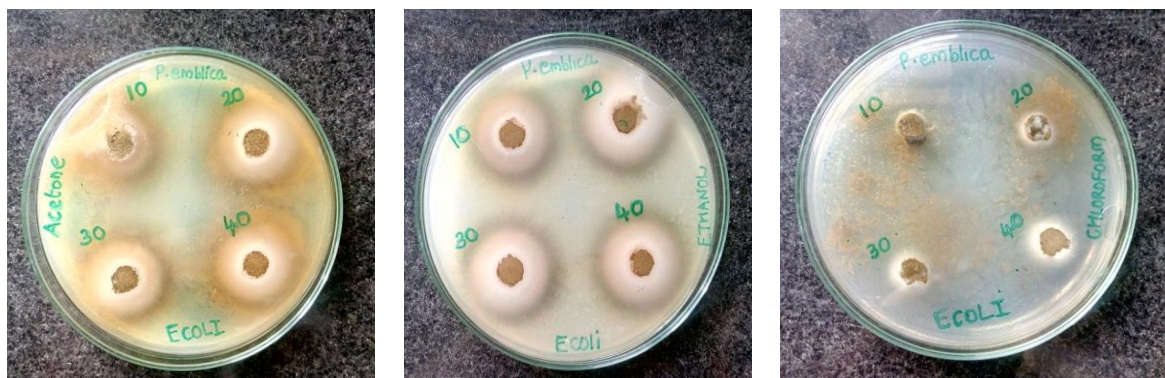
Inhibition zone diameter measured in millimeter (mm)

Followed by ethanol, acetone extract showed a sensitivity of 26.4±2.0 mm at a concentration of 40µl, thereby displaying a moderate antibacterial efficacy. And positive control showed an inhibition zone of 32.1±2.6 mm. According to the results projected by Jasna and Dhivya, [36] among the three solvent extracts of *Nigella sativa* seeds tested against the bacteria *S. typhimurium* and *S. aureus* maximum inhibitory zone was exhibited by chloroform and acetone extract respectively. Followed by acetone, the least antibacterial potential was found in chloroform extract which displayed an inhibitory zone of 12.4±0.9 mm at a concentration of 40µl. Metiner et al [37] recorded that the chloroform extraction was found to be effective for *E. coli* and *B.*

*cereus* strains relatively but only in high concentrations

**Antibacterial activity against *S. aureus***

Against the bacterium *S. aureus*, among the three extracts, maximum antibacterial potency was shown by ethanol extract of *P. emblica* leaf giving an inhibitory zone of 33.4±1.7mm at a concentration of 40µl and 30.2±1.3 mm at a concentration of 30µl respectively (Table 1 & Fig 2). Positive control showed 35.4±2.1 mm inhibition zone. Earlier studies of Agarry et al; [38] Mehrotra et al; [39] Sami et al [40] have reported antibacterial properties of ethanol extracts of *Aloe vera* against the pathogens selected. The *Aloe vera* ethanol extract showed maximum inhibitory zone against standard *S. aureus* and pronounced antibacterial activity against *K. pneumonia*.



**Fig 1: Plate showing antibacterial activity of *P. emblica* leaf extracts against *E. coli***



Fig 2: Plate showing antibacterial activity of *P. emblica* leaf extracts against *S. aureus*

Followed by the activity of ethanol was that of acetone extract which provided a zone of inhibition of  $25.8 \pm 1.0$  mm at a concentration of  $40 \mu\text{l}$ . And its positive control showed an inhibition zone of  $35.4 \pm 2.1$  mm. Minimum antibacterial efficacy among the three extracts was for chloroform extract, providing an inhibitory zone of  $11.8 \pm 0.3$  mm at a concentration of  $40 \mu\text{l}$  and it showed an inhibition zone of  $35.4 \pm 2.1$  mm in the positive control of Amoxicillin. According the research of Aqil et al; [41] Naim et al; [42] Oskay et al [43] it was interesting to note that the methanol and ethanol extracts from *tulsi*, oregano, rosemary, lemongrass, *Aloe vera* and thyme displayed antimicrobial activity to *S. aureus*. Previous studies also revealed the antibacterial potency of the investigated plant extracts and essential oils against *S. aureus*.

Overall results revealed that maximum antibacterial activity was displayed by ethanol extract followed by acetone extracts of *P. emblica* against both the test microorganisms.

### 3.2 Antioxidant activity of *P. emblica*

The results of antioxidant activity of *P. emblica* were tabulated in Table 2. Antioxidant activity was found to increase as the test concentration was increased. Therefore, among the three extracts, at the higher test concentration of  $50 \mu\text{l}$ , acetone exhibited antioxidant activity of 25.04 %. This was followed by chloroform extract with a Radical scavenging activity (RSA) of

30.54 %. Minimum antioxidant activity was displayed by that of ethanol (36.39 %) extract. In a study parallel to the present study Sumalatha [44] has observed that *P. emblica* extract showed high scavenging activity with 71.75% inhibition in comparison to the standard Ascorbic acid.

At the test concentration of  $40 \mu\text{l}$ , antioxidant activity of *P. emblica* was displayed in the order i.e., acetone (36.84 %) followed by chloroform (38.47 %) followed by ethanol (44.28 %) respectively. As the concentration decreased, antioxidant activity was also found to come down. At the lowest concentration of  $10 \mu\text{l}$  antioxidant activity of acetone was 47.54 % ethanol extract with an inhibition % of 84.02 %. Similar study was reported by Gulcin et al [45] which revealed that activity of both plants evaluated for antioxidant activity displayed that significantly higher antioxidant activity exists in the extract of *Bacopa monnieri* leaf as compared to *Centella asiatica* at different concentrations.

As presented in table the scavenging abilities of different solvent extracts of *P. emblica* were concentration dependent. Concentration of the sample necessary to decrease the initial concentration of DPPH by 50% ( $\text{IC}_{50}$ ) under the experimental condition was calculated. Therefore a lower  $\text{IC}_{50}$  value indicated a higher antioxidant activity.

Table 2: Antioxidant activity of *P. emblica* leaf extract

Treatment group	Plant extract (µl)	DPPH in methanol (µl)	Methanol (µl)	Incubation at room temperature for 30 minutes	OD at 518 Nm		
Negative Blank	-	-	1000		0.000		
Positive control	-	500	500	0.551			
				<b>Radical Scavenging Activity (% inhibition)</b>			
				PA	PC	PE	
Test concentration of Plant extracts	10	500	490	47.54	79.67	84.02	
	20	500	480	46.82	64.97	76.95	
	30	500	470	43.37	55.89	61.70	
	40	500	460	36.84	38.47	44.28	
	50	500	450	25.04	30.54	36.39	

### 3.3 Phytochemical analysis of *P. emblica* leaves

Phytochemical analysis is carried out in ethanol and acetone extracts of *P. emblica* leaves that displayed significant antibacterial and antioxidant activity. The results of phytochemical analysis of *P. emblica* leaf extract are presented in Table 3. In the present study maximum antibacterial activity was exhibited by ethanol extract of *P. emblica* against *S. aureus* and *E. coli*. The phytochemical analysis of ethanol extract of *P. emblica* showed the presence of tannins, phenols, sterols, terpenoids and proteins. In accordance to the results of present study the phytochemical screening of *Pterocarpus mildbraedii* leaves by Okwu et al [46] showed that it contained alkaloids, flavonoids, tannins, saponins. These phytochemicals exhibit a wide range of biological effect such as antibacterial and antioxidant properties.

Table 3: Phytochemicals present in *P. emblica* leaf extracts

Sl. No.	Constituents	<i>P. emblica</i> leaf	
		Acetone extract	Ethanol extract
1	Alkaloids	-	-
2	Flavonoids	+	-
3	Sterols	+	+
4	Terpenoids	+	+
5	Anthraquinones	-	-
6	Phenols	-	+
7	Saponins	+	+
8	Tannins	+	+
9	Proteins	-	+
10	Quinones	-	-

“+” Presence

“-” Absence

Moderate antibacterial activity was exhibited by acetone extract of *P. emblica* which when tested showed the presence of secondary metabolites like tannins, flavonoids, sterols, terpenoids, saponins.

Chloroform extract of *P. emblica* consist of very few phytoconstituents such as saponins, quinones, tannins and flavonoids, and showed a minimum antibacterial potential. Similar investigation of the active constituents by Onwueyiagba [47] in the leaves of *Solanum macrocarpon* showed the presence of alkaloids, flavonoids, saponins, tannins, resins and essential oil. The presence of these phytochemicals signifies the possession of medicinal properties within the leaves.

### CONCLUSION

Medicinal plants have bio-compounds which are used for curing of various human diseases and also play an important role in healing. Medicinal plants have antibacterial and antioxidant activities. The present study the leaf extracts of *P. emblica* appears to be a rich source of different phytoconstituents, with antibacterial and antioxidant compounds and also supported the statement of applicability of plant in traditional system of treatment. The result suggested that both the plants could be used as a curative agent for different ailments. In addition, phytochemicals evaluation of *P. emblica* provided information about a number of medicinally important secondary metabolites. It may be concluded from this study that ethanol and acetone extracts of *P. emblica* may be used as an important source with antibacterial and antioxidant potency.

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