

**EVALUATION OF PHYTOCHEMICAL ANALYSIS AND
ANTIMICROBIAL ACTIVITY OF *PHYLLANTHUS EMBLICA* (L.)
FRUIT PULP**

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ABSTRACT

Phyllanthus emblica (L.) is commonly known as Indian gooseberry. It has been extensively used, both as edible plants and for therapeutic potentials. The fruit of *P.emblica* have significant medicinal value. It has been used for the treatment of cancer, diabetes, liver treatment, heart trouble, ulcer, anemia and various other diseases. It has application as anti-inflammatory, anti-pyretic and gastroprotective treatments by the rural population. The present study was conducted based on the traditional knowledge of the plant. To evaluate the scientific basis for the use of amla fruit pulp there were study conducted on the isolation of the chemical constituents present in the

fruit pulp. The Phytochemical constituents of the dried powdered plant fruits were extracted using chloroform and methanol solvents, results revealed the presence of alkaloids, carbohydrates, glycosides, flavanoids, proteins, amino acids, tannins, phenolic compounds, sterols, steroids, fixed oils, fats and resins. The methanolic extract which have high phytochemical constituents used for the evaluation of antimicrobial activity at 100mg/ml, which evaluated against some common pathogenic bacteria using agar diffusion method. Gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Pseudomonas aeruginosa* were used and antimicrobial activity of the concentrated extracts was evaluated by the diameter zone of inhibition against the above microorganisms. Fruit extract were active against both stains of bacteria. The above observation indicates that *P.emblica* has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for development of new medicines with lesser side effects.

KEYWORDS: Phyllanthus emblica, Methanolic extract, Antibacterial activity, Medicinal value, Traditional knowledge.

INTRODUCTION

Herbal medicines has been practiced worldwide and is now recognized by World Health Organization (WHO) has an essential building block for primary health care (Onayude *et al.*, 1990). India with rich biodiversity ranks first for the traditional medicine depends on plant species and traditional knowledge are important of the herbal medicine trade and pharmaceutical industry where plants provide raw materials and the traditional prerequisite information. The Ayurvedic system of medicine is prevalent in India since the period and as easily as the dawn of human civilization (Nair, 1998), in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi and Mehrotra, 2002).

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing because of the presence of phytochemical constituents (Nosto *et al.*, 2000). Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root (Joseph *et al.*, 2013). Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are include in primary constituents and secondary compounds have terpenoids, alkaloids and phenols compounds (Krishnaiah *et al.*, 2007).

The emergence and spread of antibiotic resistant bacteria cause increasingly difficult therapeutic problems. Intensive research efforts have been devoted to the development of alternative antimicrobial agents to combat this problem. Historically plants have provided a source of inspiration for novel drug compounds, as plants derived medicines have made large contributions to human health and well-being. Their role is to folds in the development of new drugs or second a phytomedicine to be used for the treatment of disease. Medicinal plants being the most common source of antimicrobial agents are the part and parcel of human society to compete diseases from the down of civilization (Datar and Indap, 2006). As they have minimum side effects their usage as traditional health remedies is the most popular for 80% of world's population.

Phyllanthus emblica L. (Syn. *Emblica officinalis*) is commonly known as Indian gooseberry. It is highly nutritious and is reported as an important dietary source of vitamin C, minerals and amino acids. All parts of the plant are used for medicinal purposes, especially the fruit, which has been used in Ayurveda. The fruits of amla are widely used in the Ayurveda and are believed to increase defense against diseases. The recent study for the purpose of the research work shown that the active compounds contained by *Phyllanthusemblica* have significant medicinal value. It has its beneficial role in treatment of cancer, diabetes, liver treatment, heart trouble, ulcer, anemia and various other diseases. Similarly, it has application as antioxidant, immune modulatory, antipyretic, and analgesic, cytoprotective, diuretic, laxative, carminative and stomachic, antitussive and gastroprotective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level. It is also helpful in neutralizing snake venom and as an antimicrobial. It is often used in the form of *triphala* which is an herbal formulation containing fruits of *Phyllanthus emblica*, *Terminalia chebula* and *Terminalia bellerica* in equal proportions.

According to Ayurvedic doctors regular usage of amla will make a man live more than 100 years like a youth. Amla is supposed to rejuvenate all the organ systems of the body, provide strength and wellness. It keeps us away from all the diseases by boosting our immune system. It is believed by ayurvedic practitioners that if an individual regularly takes amla he can live up to an age of 100 without suffering from any type of ailment.

Considering all the above mentioned uses of *Phyllanthus emblica* an attempt was made to study with the following objectives.

- To analyse the phytochemical constituents of *Phyllanthus emblica* fruit pulp in chloroform and methanol extract.
- To analyse the antimicrobial activity of *Phyllanthus emblica* fruit pulp in methanol extract.

MATERIALS AND METHODS

Collection of plant materials

The plant species *Phyllanthus emblica* is an important medicinal herb. The fresh plants were collected from Calicut district of Kerala, during November 2018.

Shade dry of the collected plant materials

The selected fruit varieties of *P.emblica* were cleaned to remove adhering dust and then dried under shade. Finally powdered with the help of a pulverizer. This powder was used for further studies.

Phytochemical Studies

Extraction

The fruit powder of different varieties was subjected to methanol, chloroform and water. 45gm of each variety was extracted using shaker apparatus and this was used for further analysis.

Phytochemical analysis of extract

Phytochemical analysis of extracts of the plant was carried out and their bioactive compounds were determined by the following methods Harbone (1973) and Koktae, *et al.*, (1995).

ANTIMICROBIAL SUSCEPTIBILITY TEST

Media Used

Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water Initially, the stock cultures of bacteria *P.aeruginosa* and *S.aureus* were inoculating in broth media and grown at 37°C for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hold cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different volumes. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zone were noted.

P.Emblica Fruit Pulp Methanolic And Chloroform Extracts



RESULTS

Phytochemical Evaluation

Qualitative phytochemical analysis of chloroform and methanolic extracts of *P.emblica* were presented in (Table 1). Chloroform extracts of *P.emblica* showed the presence of alkaloids, saponins, carbohydrates, glycosides, flavonoids, proteins and aminoacid, tannins and phenolic compounds, oils, fats and resins. But steroids and terpenoids are completely absent. In Methanolic extracts alkaloids, carbohydrate, flavonoids, proteins and aminoacids, tannins and phenolic compounds, steroids and resins are present. Saponins, glycosides, oils and fats and terpenoids are completely absent.

1. Test for carbohydrates

To 2 ml of test solution adds two drops of the Molish reagent (a solution of α naphthol in 95% ethanol). The solution is then poured slowly in to a test tube containing 2 ml of conc. Sulphuric acids so that two layers form. The formation of a purple product at the interface of the two layers indicates the presence of carbohydrate.

2. Test for proteins

It is used to determine the presence of peptide bonds in protein. To 3 ml of test sample is add 3% NaOH and a few drops of 1% CuSO_4 . The solution turns from blue to violet (purple) or to pink. That indicates the presence of protein.

3. Test for starch

Mix 3 ml of test solution and few drops of dilute iodine solution. Blue color appears. It disappears on cooling and reappears on heating.

4. Test for amino acids

To 5ml of test sample solution is add a few drop of 40% NaOH and 10% of lead acetate and boiled the solution formation of black precipitate show the presence of amino acids.

5. Test for steroids

To 2 ml of extract and add 2 ml of chloroform and add 2 ml conc. Sulphuric acid. Shake well, chloroform one layer appear and acid layer show greenish yellow florescence which indicate the presence of steroids.

6. Test for glycosides

To the solution of extract add glacial acetic acid, few drops 5% ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and bluish green color in upper which indicates presence of glycosides.

7. Test for flavonoids

To 2 ml of extract and few drops of 1% of Ammonia solution. A yellow coloration is observed for the presence of flavonoids.

8. Test for alkaloids

To 0.5g of each extracts adds 5ml of 1% of aqueous hydrochloric acid and kept in water bath: 1ml of the filtrate is to be treated with Mayer's reagent (Potassium iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

9. Test for tannins

To 0.5ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and black color for catecholic tannins.

10. Test for saponins

To 1 ml of extract solution, 1 ml of water and shake it. Persistent foam indicates presence of saponins.

11. Test for terpenoids

2ml extract was mixed with 2ml of chloroform in a test tube. To this 3ml of conc. Sulphuric acid was added along the walls of the tube to form a layer. An interface with a reddish brown coloration confirmed the presence of terpenoids.

12. Test for gums

To 1 ml of extract add 3 ml of Dil. Hcl solution is added drop by drop till red coloration visualizes the presence of gums.

RESULTS**Phytochemical Evaluation**

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phenolic compounds, oils, fats and resins. But steroids and terpenoids are completely absent. In Methanolic extracts alkaloids, carbohydrate, flavonoids, proteins and aminoacids, tannins and phenolic compounds, steroids and resins are present. Saponins, glycosides, oils & fats and terpenoids are completely absent.

Antibacterial Activity

Antibacterial activity of *P.emblica* is given in the (Table 2). Methanolic extract of *P.emblica* shows high activity against the pathogens than standard antibiotic activity against pathogens. In this 100mg/ml of methanolic extract given maximum activity against two strains of bacteria, that are against *P. aeruginosa* and *S.aureus* (Table 2) (Fig 1). In this highest activity shown against *P. aeruginosa*. Antimicrobial test done with a standard antibiotic named Ciprofloxacin show lower zone of inhibition and less activity against two same strains of bacteria (Table 3) (Plate 4).

Table 1: Phytochemical Screening of *P.Emblica* Fruit Pulp.

Sl.No.	Phytochemicals	Crude extract of <i>P.emblica</i>	
		Chloroform	Methanol
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Saponins	-	-
4	Cardiac glycosides	+	-
5	Terpenoids	-	-
6	Sterols and Steroids	-	+
7	Fixed oils and Fats	+	+
8	Resins	-	+
9	Carbohydrates	+	+
10	Proteins and Amino acids	-	+
11	Tannins and Phenolic compounds	+	+

Phytochemical Screening of *P.Emblica* Fruit Pulp



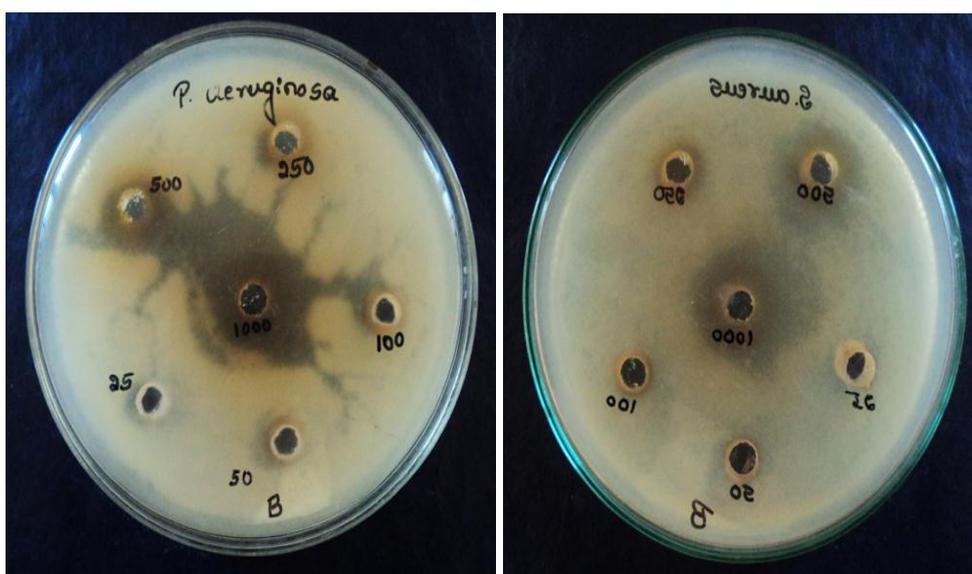
Table 2: Antibacterial Activity of Methanolic Extract of *P.Emblica* Fruit Pulp.

Sl.No.	Name of the bacterial species	Zone of inhibition (mm)					
		1000	500	250	100	50	25
1	<i>P.aeruginosa</i>	20	10	3	3	0	0
2	<i>S.aureus</i>	18	15	3	0	0	0

Table 3: Standard Antibiotic (Ciprofloxacin).

Sl.No.	Name of the bacterial species	Zone of inhibition (mm)					
		1000	500	250	100	50	25
1	<i>P.aeruginosa</i>	14	8	3	1	0	0
2	<i>S.aureus</i>	34	27	25	21	18	13

Antibacterial activity of methanolic extract of *P.emblica* fruit pulp



Antibacterial activity of standard antibiotic



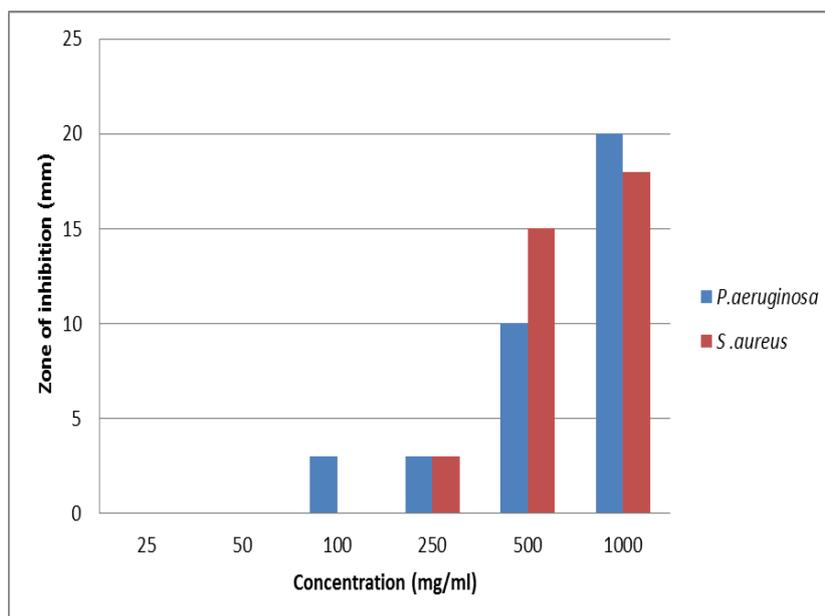


Figure 1: Antibacterial activity of methanolic extract of *p.emblica*.

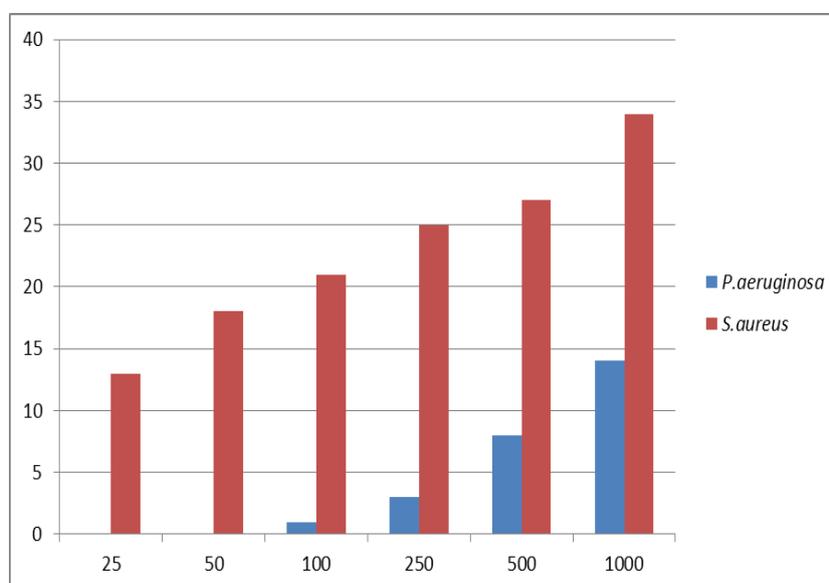


Figure 2: Antibacterial activity of standard Antibiotic.

DISCUSSION

The importance of medicinal plants in providing healthcare against various ailments including infectious diseases is well documented. Plants extracts have been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to microbial activities. The development of resistance to antibiotics is an almost inevitable consequence to their application. The speed of resistance depends on the respective class of antibiotics and their product use. For many years, medicine depended exclusively on leaves, flowers and barks of plants, Only recently have synthetic drugs come into use and in many

instance, these are carbon copies of chemical identified in plants. In an overview of a bioactive data obtained from the current investigation, it can be highlighted that the tested extracts have potential to inhibit bacteria.

The present study revealed the presence of alkaloids, carbohydrate, flavonoids, proteins and amino acids, tannins and phenolic compounds, steroids and sterols, resins in methanolic extract of *P.emblica* fruit pulp than in chloroform extract. Saponins are completely absent in both.

Similar studies have been reported by Raghu and Ravindra, (2009), stated that *P.emblica* methanolic extract exhibited a significant antimicrobial activity. The methanolic extract show more antimicrobial activity than the chloroform and diethyl ether extracts.

Dhale and Mogle, (2011), Amir *et al.*, (2011) reported preliminary phytochemical screening revealed that the extract of *P.emblica* possesses flavonoids, steroids and phenolic compounds. This result indicated that methanolic extract possesses antioxidant property.

In the present study out of the two solvents, Methanolic extracts was selected for antimicrobial test and it showed very high inhibitory actions towards bacteria. In this solvent inhibitory action against both +ve and -ve strains of bacteria only 1000mg/ml of the extracts has given maximum compare to other concentration. The methanolic extract of *P.emblica* shows high inhibitory action against bacteria than antibacterial activity of standard antibiotics. The antimicrobial activity between extract obtained with methanol and antimicrobial activity of standard antibiotic shows results that the methanol was more active when compare with antibiotic activity. The inhibitory activity may be pathogen specific or due to the phytochemical properties of respective plant species and the solvents used for the extraction of secondary metabolites. The chemical constituent present in the plant are responsible for the antimicrobial activity.

Analysing the phytochemicals with antimicrobial activity in *P.emblica* fruit pulp will provide scientists with insight into how effective these *P.emblica* fruits in terms of its medicinal value which can lead to the development of new medicine.

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