



Evaluation of Antibacterial and Antifungal Activity of *Phyllanthus emblica* Leaf Extract.

N. Malliga Elangovan^{1*}, M.S. Dhanarajan², I. Elangovan³

¹ Research Scholar, Department of Biochemistry, Manonmaniam Sundaranar University, Tirunelveli.

² Principal, Jaya College of Arts and Science, Thirunindravur, Chennai.

³ Biochemist, Meenakshi Mission Hospital, Madurai, Tamil nadu.

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*Corresponding author:
malligain@gmail.com

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Abstract

Objective: *Phyllanthus emblica* is a well known medicinally valued plant. Although the efficacy of *P.emblica* fruit is widely proved, the use of its leaf is less investigated. The main objective of the present work is to evaluate the antimicrobial activity of *P. emblica* leaf extract against some human pathogens responsible for severe illness. **Methods:** This study was subjected to investigate the antimicrobial properties of petroleum ether leaf extract of *Phyllanthus emblica* by disc diffusion method. The minimum inhibitory concentration of antimicrobial activity of the *P. emblica* extract at a concentration ranging from 1000 µg to 62.5 µg was compared with the standard drug Streptomycin (disc 10 µg) and Amphotericin B (20 µg). **Result:** This study revealed that the petroleum ether leaf extract of *Phyllanthus emblica* has potential antibacterial and antifungal activity against all the tested strains except *Enterococcus faecalis*. **Conclusion:** This study concludes that *Phyllanthus emblica* leaves have broad spectrum of antimicrobial activity and a potential source of new classes of antibiotics that could be useful in chemotherapy and control on human infectious diseases.

KEYWORDS: *Phyllanthus emblica*, petroleum ether extract, Antibacterial Activity, Antifungal Activity, Zone of inhibition.

INTRODUCTION

In our modern days, the rate of infection with antibiotic resistant micro organism has been increased. Therefore, actions must be taken to reduce this problem to continue studies for the development of new drugs, either natural or synthetic. Since ancient period, many traditional herbs are used in the treatment of various diseases. The knowledge of plants has formed the basis of sophisticated traditional medicine systems and continues to provide mankind in the development of new remedies [1]. Medicinal plants are rich source of anti microbial agents and its therapeutic uses are becoming popular because of its lesser side effects, economic and are easily accessible to humans [2].

Phyllanthus emblica is a medium-sized deciduous tree, belong to Euphorbiaceae family. It is highly nutritious and vital dietary source of vitamin C, carotene, thiamine, riboflavin, niacin, amino acids, and minerals such as calcium, phosphorus, iron [3, 4]. In *P. emblica* fruit, vitamin C is considered to be highly stable due to the presence of tannin and polyphenols [5]. The fresh or dry fruit is widely used for the treatment of diarrhoea, jaundice and inflammatory disorder.

S. Saeed and P. Tariq, (2007) found that aqueous infusion of *P. emblica* exhibit potent antibacterial activity against *E. coli*, *K. pneumoniae*, *K. ozaenae*, *Proteus mirabilis*, *P. aeruginosa*, *S. typhi*, *S. paratyphi* A & B, and *Serratia marcescens*, but did not show any antibacterial activity against some Gram-negative urinary pathogens [6]. In a study, the chloroform extract of fresh ripe fruit of *P. emblica* confirmed the strongest inhibitory effect against *Bacillus subtilis* and moderate inhibitory activity against *Bacillus cereus*, *S. typhi*, *Shigella dysenteriae*, *S. aureus*, *E. coli*, *S. paratyphi*, *P. aeruginosa*, *Vibrio parahaemolyticus* and *V. mimicus* [9]. The fruit of *emblica* is widely used in researches as an immunity booster, lower blood cholesterol, enhances memory and intelligence, a natural source of vitamin c and iron [8, 9] but the use of leaf is less investigated.

E.coli is a gram negative bacteria can cause UTI, bloody urine and diarrhea. *Staphylococcus aureus* is a gram-positive bacterium which commonly colonises human skin and mucosa (e.g. inside the nose) without causing any harm. However, in case of trauma or surgery, *S. aureus* can enter the underlying tissue, forming its characteristic local abscess lesion. Entry of this pathogen into the lymphatic channels or blood can cause septicaemia [10].

Aspergillus niger is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and, in severe cases, damage to the ear canal and tympanic membrane. *Candidiasis* is a common infection of the skin, oral cavity, oesophagus, gastrointestinal tract, vagina and vascular system of humans. The infections are commonly seen among immunocompromised patients. *Candida albicans* is the organism most often responsible for disease which expresses numerous virulence factors that contribute to pathogenesis [11].

As the medicinal plants are getting hold of more importance in pharmaceutical industries for the preparation of new phytomedicines, study was undertaken to check its properties as a drug [12]. In the present investigation, the petroleum ether leaf extract of *Phyllanthus emblica* was subjected to antibacterial and antifungal activity assays.

MATERIALS AND METHODS

(i) Plant Collection

The leaves of *Phyllanthus emblica* were collected from Algar kovil hills, Madurai, Tamil nadu, India. The selected plants were authenticated by Dr. P. Jayaraman, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai- 05. The collected plant leaf sample was washed thoroughly under running tap water to remove dust and sand particles. It was then shade dried for a week to avoid loss of essential oil, powdered with the aid of grinding machine and stored for further use.

(ii) Preparation of Plant extract

In order to extract the active compounds from plant leaves, 200 g of each dried sample was extracted in Soxhlet apparatus with 250ml of petroleum ether for 8 hours and filtered using Whatmans No. 1 filter paper. The extract obtained was evaporated completely for dryness using rotary vacuum evaporator to give a concentrated extract at 60°C in a water bath. It was then dried aseptically with the help of drier and subjected to antimicrobial analysis.

(iii) Microbial Strains Used:

The crude extracts of the plant and drugs were tested for antimicrobial activity against few strains of human pathogenic microbes. Antibacterial effect of *Phyllanthus emblica* leaf was determined by using five different bacterial strains namely *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Salmonella typhi* and *Escherichia coli*. Antifungal effect of *Phyllanthus emblica* was determined against three different fungal strains viz. *Aspergillus niger*, *Candida albicans*, and *Penicillium notatum*. The pathogenic strains were obtained from Bose Clinical Laboratory, Madurai- 625 001.

(a) Antibacterial Screening

The antibacterial activity was determined by disc diffusion method. A loop of bacteria from the agar slant stock was cultured in nutrient broth over night and spread with a sterile cotton swap into petri plates containing 10 ml of Muller Hinton agar (MHA) medium. Sterile filter paper discs impregnated with the 20 µl of petroleum ether leaf extract (Concentration: 1000 µg, 500 µg, 250 µg, 125 µg & 62.5 µg) were kept on the cultured plates and incubated at 37°C for 24 hours. DMSO (20 µl) is the solvent without plant extract served as negative control. Standard antibiotic Sterptomycin (disc.10 µg) was employed as positive control. The plates were kept for half an hour for pre incubation diffusion. Then the plates were kept for incubation at 37°C for 24 hours. After incubation, antibacterial activity was assessed by measuring the diameters of inhibition zone. The diameters of the zones of inhibition by the samples were then compared with the diameters of the zones of inhibition produced by the standard antibiotic discs.

(b) Antifungal Screening

Three different species of fungal pathogen were maintained in Potato Dextrose Broth (PDB) for 24 hours. In vitro antifungal activity was determined by using disc diffusion technique. The fungal pathogens were inoculated by spread plate method using 0.1 ml of 24 hours old culture by sterile swabs. Whatman No.1 filter paper discs were sterilized and impregnated with 20 µl of *P. emblica* extract (Concentration: 1000µg to 62.5 µg). Amphotericin B (20 µg) used as positive control and DMSO (20 µl) kept as negative control. The fungal petri plates were incubated for two days at 37°C, then plates were observed and the diameter of zone of inhibition was measured in milli meters (mm).

RESULTS

Medicinal plants are being explored as an alternate source to get therapeutic compounds on the basis of their medicinal properties. Active petroleum ether extracts of *P.emblica* have been shown to possess antimicrobial properties against a variety of pathogens.

Antibacterial activity

The petroleum ether extract of *P.emblica* leaf sample exhibited antibacterial activity against all the test pathogens except *Enterobacter feacalis* (Figure-1). Plant extract at 1000 µg concentration showed the highest zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* (11 mm) with maximum antibacterial activity, whereas moderate activity against *Salmonella typhi* (9 mm), comparatively less activity on *Bacillus subtilus* (8 mm) and no antibacterial activity against *Enterobacter feacalis*. The plant extracts have shown high antimicrobial activity towards Gram positive as well as Gram negative bacteria (Table-1).

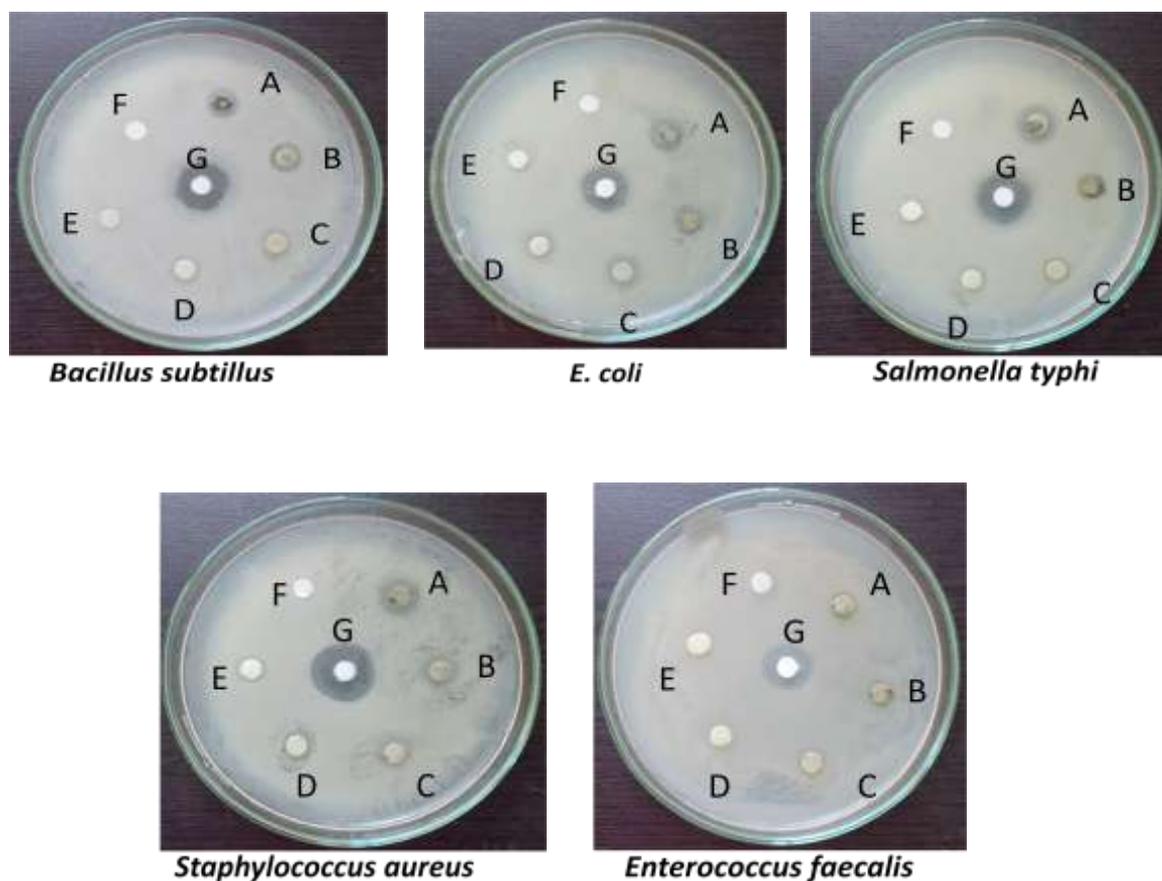


Figure-1: Diameter of Zone of inhibition of *Phyllanthus emblica* leaf extract

Fig Label: A= 1000 µg, B=500 µg, C = 250 µg, D=125 µg, E=62.5 µg, F= DMSO (-ve control), and G = Standard (+ve control)

Table- 1: Zone of inhibition (mm) by disc diffusion method

Organisms	Concentration of <i>Phyllanthus emblica</i> leaf extract					Antibiotic
	1000 μ g	500 μ g	250 μ g	125 μ g	62.5 μ g	Streptomycin (10 μ g)
<i>Staphylococcus aureus</i>	11	9	7	7	6	16
<i>Bacillus subtilis</i>	8	8	2	0	0	15
<i>E. Coli</i>	11	9	9	2	0	15
<i>Salmonella typhi</i>	9	7	7	7	0	15
<i>Enterococcus faecallis</i>	0	0	0	0	0	14

Anti fungal activity

The results revealed that the plant leaf extract possess high inhibitory activity against the fungi (Figure- 3).

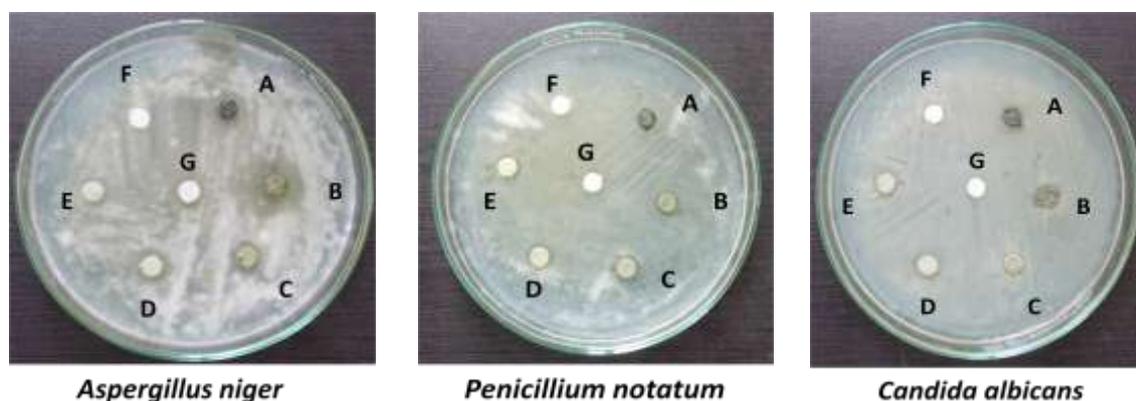


Figure-3: Anti fungal activity of *Phyllanthus emblica* leaf extract.

Fig Label: A= 1000 μ g, B=500 μ g, C = 250 μ g, D=125 μ g, E=62.5 μ g, F= DMSO (-ve control), and G = Standard (+ve control)

Petroleum ether extract of leaf sample (500 μ g) showed highest anti- fungal activity against *Aspergillus niger* (17mm) whereas *Candida albicans* and *Penicillium notatum* showed minimum zone of inhibition (6mm) at different concentrations (Figure-4).

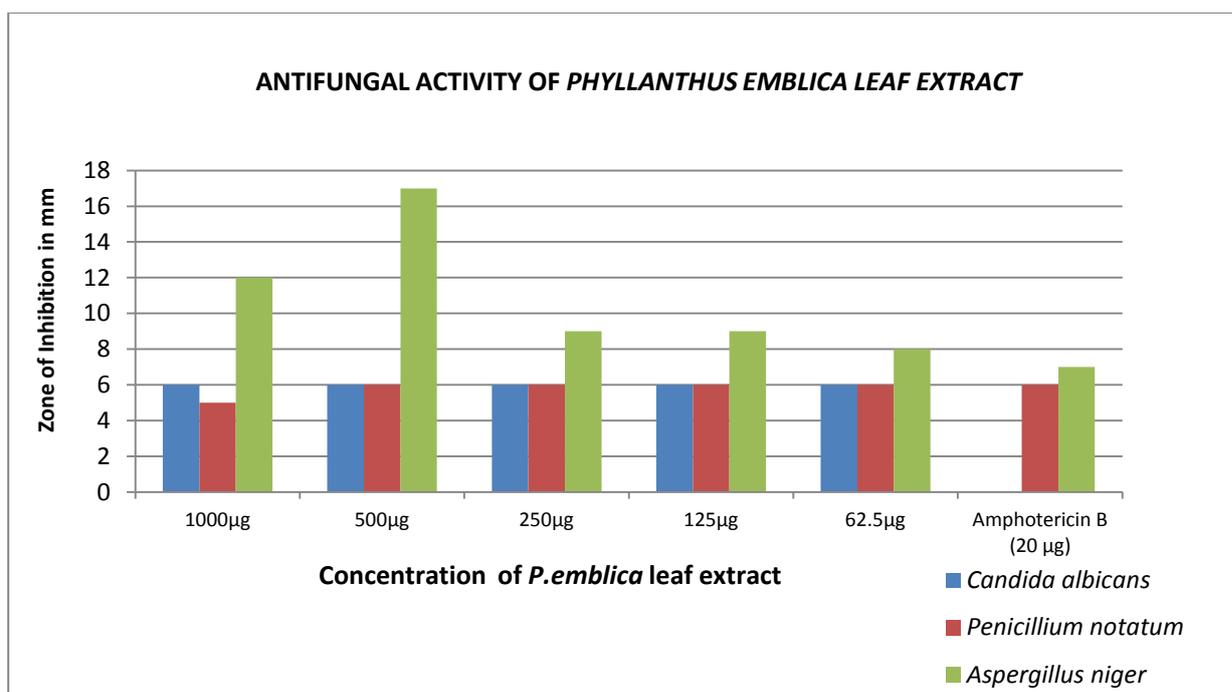


Figure-4: Zone of inhibition (mm) by disc diffusion method

DISCUSSION

The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. Crude petroleum ether leaf extract of *P. emblica* have a significant antimicrobial activity against broad spectrum of micro organisms with the exception of *Enterobacter faecalis*, indicating that active ingredients in plant materials could be extracted into petroleum ether. No inhibition was observed with controls, which proves that solvents could not act as antibacterial agents. Highest antibacterial activity was observed against *Staphylococcus aureus* and *E. coli* with the indication that the leaf extract is beneficial as a cure for skin diseases, and urinary tract infection (UTI).

In traditional Indian medicine, all parts of this plant are used in various herbal preparations. Ancient knowledge together with scientific principles can provide us with potent remedies to eradicate the diseases. An infusion of the *Phyllanthus* leaves with fenugreek seed is given for chronic diarrhoea [13]. Highest anti fungal activity against *Aspergillus niger* prove the potential of the plant in preventing diarrhoea and also function as an anti-allergic in nature. In contrast to this result, the *Phyllanthus* extract did not exhibit any antifungal activity against *A. niger* at the condition studied by Varaprasad et al [14].

In a previous study, *Phyllanthus emblica* leaves demonstrated the presence of seventeen bioactive compounds namely alcohol, saturated hydrocarbons, unsaturated fatty acid, fatty alcohol, alkane hydrocarbons, vitamin E, ester compounds, plant sterols and triterpenes which may acknowledge the medicinal property of this plant. Thus the antimicrobial activity of this plant must be due to the presence of the secondary metabolites such as 1-Hexacosanol, Octadecanoic acid, Methyl ester, Gamma Sitosterol, and 12-Oleanen-3-yl acetate [15].

The microbial studies of the leaf extract showed significant antimicrobial activity indicating the potential for the discovery of novel drugs as a remedy for different bacterial and fungal diseases. Further research is necessary for successful separation, purification and characterization of individual biologically active compounds present in leaf extracts of *P. emblica* using column chromatographic methods and spectroscopic techniques. Characterisation of the secondary metabolites in medicinally important plants has a vital role to prove as an effective anti microbial drug to extend as cost effective formulation.

The antimicrobial activities of bioactive compounds may be due to one or more possible means of actions. For example, oil degrades the cell wall, interact with the cell membrane composition and disrupt the plasma membrane [16], damage membrane protein, change fatty acid and phospholipid constituents, obstruct the membrane integrated enzymes, impair enzymatic mechanism for energy coagulate cytoplasm, cause leakage of cellular components, diminish the proton motive force, change nutrient uptake and electron transport, etc [17].

CONCLUSION

Based on the result of this study, it is concluded that *Phyllanthus emblica* leaves proved to have rich source of valuable medicinal compounds to be a potential antibacterial and antifungal properties. These finding support the use this plant for folk medicine to treat and control many diseases. The bio active secondary metabolites shall be further explored for the isolation and purification of its biologically active compound for clear understanding of the mechanism of action. These findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new effective and economic drugs to prevent various human diseases.

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