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Research Article

DRUGS RESISTANCE AND SCREENING OF ANTIBACTERIAL ACTIVITIES OF *PHYLLANTHUS EMBLICA* (L) FRUITS PRESERVING PROTIENS

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Abstract

The fruit of Emblica officinalis commonly known as amla is highly valued in traditional Indian medicine. *Phyllanthus emblica* (L) has been used for the anti-inflammatory and anti-pyretic treatments by the rural population. *P. emblica* has been used for the treatment of several. Based on the traditional knowledge about the plant, present study was conducted. To evaluate the scientific basis for the use of plant, the antimicrobial activities of the extracts at different doses (10mg and 20mg/ml) of the leaves and fruits were evaluated against some common pathogenic bacteria using agar disc diffusion method. Gram positive bacteria like *staphylococcus aureus* and *Bacillus subtilis* gram negative bacteria like *Pseudomonas aeruginosa, and Escherichia coli* were used and antimicrobial activity of the concentrated extracts was evaluated by the diameter zone of inhibition against the above microorganisms. Plant extracts were active against both gram positive, gram negative 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 infectious disease chemotherapy and control. The study also conducted on the isolation of the chemical constituents present in the plant.

Keywords: Antibacterial activity, *Phyllanthus emblica*, Traditional knowledge, Medicinal plants

Introduction

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, 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 are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Nepal is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In Nepal thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75 - 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele 1993).

P. emblica Linn. (Euphorbiaceae), a tree growing in subtropical and tropical areas of Far- Eastern countries has been reported to contain constituents with variable biological effects. The activities of crude leaf extracts were evaluated in human polymorphonuclear leukosytes (PMNs) and platelets.

The study showed that the plant leaves have antineutrophil and antiplatelet properties *in vitro.* This agrees with the anti-inflammatory

and antipyretic usage of this tree in traditional medicine by rural populations in Asia. The anti- inflammatory activity of a number of *P. emblica* L. leaf extracts was measured in order to verify the traditional uses of this ethnomedical plant (Summanen 1999).

P. emblica has been used for the anti-inflammatory and anti-pyretic treatments by the rural population. *P. emblica* has been used for the treatment of several disorders such as the Scurvy, Cancer and Heart diseases. The important constituent of plant leaves have the anti- neutrophilic activity and anti-platelet properties *in vitro.* The extracts also possess several pharmacological properties like anti-viral (HIV, AIDS, HERPES VIRUS, CMV) antimutagenic, antiallergic, anti-bacterial activities (Khopde *et al* 2000). *P. emblica* L contains different class of secondary metabolites (Calixto *et al* 1998).

It has been used for anti-inflammatory and antipyretic treatments by rural populations in its growing areas. Malays use a decoction of its leaves to treat fever (Burkill 1966). In Indonesia, the pulp of the fruit is smeared on the head to dispel headache and dizziness caused by excessive heat (Perry 1980). The earlier chemical findings and biological activities have since been confirmed with more advanced techniques. Active principles or extracts of *P. emblica* L. have been shown to possess several pharmacological actions, *e.g.* analgesic, antiinflammatory, antioxidant, chemoprotective, hypolipidaemic and anti-HIV-1 (Human immunodeficiency virus-1) activities (Summanen 1999).

Material and Methods

Sample collection and Authentication: The fresh, mature healthy leaves and fruits of *Phyllanthus emblica* Linn. (Euphorbiaceae) were collected from Farm of Dhule away from pollution. The plant materials were identified using the Flora of Dhule and Nadurbar District (Patil 2003) at Post-graduate Department of Botany, SSVP Sansthas, L.K.Dr.P.R. Ghogrey Science College, Deopur, Dhule-424005 (M.S.) India.

Sample preparation: Fully grown leaves and fruits of *P. emblica* were weighed (1kg). The fruits were peeled off and cut in to small pieces and shade dried. Then shade dried leaves and small pieces of fruits were ground and sieved with 2mm copper sieve to form uniform powder and stored in airtight bottles.

Preparation of extract: The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; petroleum ether, chloroform, and alcohol (Vogel 1988). The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40°C) with the help of heating mantle. The sticky greenish- brown substances were obtained and stored in refrigerator for prior to use (Beyer and Walter 1997). Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods (Harborne 1984; Trease and Evans 1987; Ajaiyeoba 2000; Edeoga *et al* 2005). The positive tests were noted as present (+) and absent (-).

Preparation of microorganism: Isolation of bacterial species of Gram positive *(Staphylococcus aureus* and *Bacillus subtilis)* and Gram negative *(Echerichia coli* and *Pseudomonas aeruginosa)* takes place. The cultures of these bacteria were checked for purity by doing gram staining and biochemical test and they were grown in nutrient broth at 37°C and maintained in nutrient agar slants at 2-8°C. Nutrient agar medium was used as bacterial culture medium in the antibacterial assays.

Dilutions and Inoculum preparations: The dried plant extracts of *P. emblica* and antibiotic Amphicillin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 10, 20 mg/ml. The inoculums of *Staphylococcu aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* were prepared in nutrient broth medium and kept incubation at 37°C for 8 hours. After growth was observed, the cultures are stored in the refrigerator at 2-8°C for analysis.

Procedure for performing the Disc Diffusion method: The required amount of Petri plates is prepared and autoclaved at 121°C for 15 minutes. They were allowed to

cool under laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dish and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with different concentrations of about 10, 20mg/ml of plant extract of *P. emblica* and antibiotic Amphicillin into each separate disc of about 40 µ1. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm).

RESULTS

The different solvent extract of *Phyllanthus emblica* of leaves and fruits showed antibacterial activity against all the test organisms. Petrolieum ether, chloroform and alcohol extracts of *P. emblica* leaves and fruits were tested against various Gram-negative and Gram-positive bacteria (Table 1, 2). Among the extracts assayed, the alcohol leaf extracts of *P. emblica* exhibited good activity against *S. aureus* at 20 mg/ml for example, 22 mm was recorded as diameter zone of inhibition. This was followed by 14 mm *B. subtilis*, 12 mm *E. coli* and *P. aeruginosa* 11 mm respectively. The least activity of bark is 3 mm against *E. coli, 4mm* for *P. aeruginosa* and *B. subtilis*, whereas 5 mm of *S. aureus* at 10mg/ml was recorded by petroleum ether extracts (Table 1). The fruit extracts (Table 2) of *P. emblica* exhibited superior activity against *S. aureus* at 20mg/ml for example, 29 mm was recorded as diameter zone of inhibition. This was followed by 18 mm *B. subtilis*, 15 mm *P. aeruginosa* and *E. coli* 12 mm respectively. The least activity of bark is 4 mm against *P. aeruginosa*, 5 mm for *B. subtilis* and *E. coli* whereas 8 mm for *S. aureus* at 10mg/ml was recorded by petroleum ether extracts. Activities of the various extracts were comparable to those of standard antibacterial agent Ampicillin.

Table1: Antibacterial efficacy of different solvent extracts of *Phyllanthus emblica* leaves

S.No.	Microorganism	Strain +/-	Concentration(mg/ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Alcohol	Amphicillin (40 (mg/ml)
1.	Escherichia coli	-ve	10	03	04	06	17
			20	07	09	12	
2.	Pseudomonas aeruginosa	-ve	10	04	05	06	22
			20	07	08	11	
3.	Staphylococcus aureus	+ve	10	05	08	12	30
			20	10	13	22	
4.	Bacillus subtilis	+ve	10	04	06	07	26
			20	09	10	14	

Table2: Antibacterial eficacy of different solvent extracts of *Phyllanthus emblica* Fruits

S.No.	Microorganism	Strain +/-	Concentration (mg/ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Alcohol	Amphicillin (40 ng/ml)
1.	Escherichia coli	-ve	10	05	05	08	18
			20	09	10	12	
2.	Pseudomonas aeruginosa	-ve	10	04	05	09	23
			20	10	11	15	
3.	Staphylococcus aureus	+ve	10	08	12	20	30
			20	12	17	29	
4.	Bacillus subtilis	+ve	10	05	08	10	25
			20	09	14	18	

DISCUSSION

Naturally occurring substance of plant origin have been reported to inhibit the growth of microorganisms. 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 (Irobi 1992). Bacterial infection seems especially controllable due to good hygiene and the availability of effective antibacterial drugs. The development of resistance to antibiotics is an almost inevitable consequence of their application (Ekhaise and Okoruwa 2001). 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 orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients are extracted, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food (Conway 1973).

Phyllanthus emblica L has been used for the anti-inflammatory and antipyretic treatments by the rural population in its growth areas in India. It is one of the common ingredients of many ayurvedic medicines. It is consumed as vegetable in pickles and other dishes in India. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

In conclusion, all of the plant extracts tested in this study had potential antibacterial activities against the reference strains.

Our results support the use of these plants as traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search of new drugs. Further studies are ongoing to isolate, identity, characterize and elucidate the structure of the bioactive components.

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