ANTIMICROBIAL ACTIVITY OF *PHYLLANTHUS EMBLICA*

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ABSTRACT

Phyllanthus emblica (Indian gooseberry or Amla) is the most celebrated plant in the Indian traditional system of unani and ayurvedic medicine. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections. Among the nearly 15,000 flowering plants documented, many of them are used as sources of medicine. In the developing nations, almost 80% people depend on these plants for medicine because of their easy availability and low cost of treatment. The modern allopathic system of medicine is known to produce serious side-effects and resistance against antibiotics which make these drugs non-potent. A large number of secondary metabolites such as tannins, alkaloids, phenolics and terpenes are responsible for the valuable pharmacokinetic properties of medicinal plants and nucleic acids. Medicinal plants are indeed the most important source of life saving drugs for the majority of the world's population. In the present manuscript we are studying the antimicrobial activity *Phyllanthus emblica*

Keywords: *Phyllanthus emblica, Indian gooseberry*

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INTRODUCTION

The use of medicinal plants as a therapeutic aid for alleviating human ailments can be traced back to over five millennia. Present day antibacterial therapy is mainly focussed on the administration of antibiotics and synthetic drugs. The indiscriminate use of antibacterial, particularly the antibiotics, has resulted in the emergence of resistant microbial strains and accumulation of metabolites in tissues and fluids, finally culminating in toxicity and adverse side effects(Noor et al., 2004). The increased demand for effective, and more safe therapeutics has resulted in the renewed interest for use of natural products in improving health and fitness. Medicinal plants are rich sources of bioactive compounds such as alkaloids, flavonoids and phenolic compounds (Kannan et al., 2009). Herbs and spices have been an important constituent in human diet since time immemorial. Besides boosting up flavour 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 anti pyretic 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. Amla is one of the richest natural sources of vitamin C, its fresh juice containing nearly twenty times as much vitamin C as orange juice. Clinical tests on patients suffering from pulmonary tuberculosis have shown that this high concentrate is more quickly assimilated then the synthetic vitamin. It is an ingredient of many Ayurvedic medicines and tonics, as it removes excessive salivation, nausea, vomiting, giddiness, spermatorrhoea, internal body heat and menstrual disorder. 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 antineutrophilic activity and anti-platelet properties in vitro. The extracts also posses 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). In the present work we are concentrating the effect of plant extract on human pathogens such as Pseudomonas aeruginosa, Bacillus amiloliquifaciens, Staphyllococcus aureus and Eschirichia coli.

Material and Methods

To perform the study of antimicrobial activities of sample medicinal plants Coriandrum sativum the plant samples were collected from Jayanti Kunj Garden, Rewa, M.P. and bacterial strains Pseudomonas aeruginosa, Bacillus amiloliquifaciens, Staphyllococcus aureus and Eschirichia coli were taken from Department of Applied Microbiology, University of Sagar, M.P.

Multiple drug resistance Culture preparation

120 ml of nutrient broth was prepared and poured in each conical flask. The broth was then autoclaved and after autoclaving they were left to cool at room temperature in laminar air flow chamber. 100µl each of Pseudomonas aeruginosa, Bacillus amyloliquifaciens, Staphylococcus aureus and Escherichia coli were inoculated into the four flasks. The inoculated culture was then kept in shaker overnight for growth.

Plant Extract preparation

Washing and drying of all the sample

Plants leaves were washed with distilled water and dried in hot air oven for 1-2 days to reduce the moisture content. Each of dried plant samples were weighed 4.00gm, and then crushed in 70% ethanol in the ratio of 1:8 in the mortar pestle and grinded properly then crushed samples were filtered through whattman filter topic 1 in a flask/beaker. Filtrates were placed in hot air oven at 40°C in a flask/beaker till it completely dry for 2-4 days. Dried filtrate was dissolved in 5ml 0f 1X tris saline buffer and stored in refrigerator.

Preparation of agar plates

Nutrient Agar media was prepared and autoclaved then it was poured in autoclaved petriplates, then it was left for 15-20 minutes to solidify.50 µlitre of culture (Pseudomonas aeruginosa, Bacillus amiloliquifaciens, Staphylococcus aureus and Escherichia coli) were spread it into nutrient agar plates respectively.

MDR with standard drugs

Here, to get the standard reference values, the tetracycline, chloramphenicol drugs were taken. Different concentration (25, 50 and 75 µg) of these drug's are poured into the wells of Pseudomonas aeruginosa, Bacillus amiloliquifaciens, Staphylococcus aureus and Escherichia coli plates respectively.

Testing with plant sample

In order to check the antimicrobial activity against selected microbes (Pseudomonas aeruginosa, Bacillus amiloliquifaciens, Staphylococcus aureus and Escherichia coli), three wells were made in each of the culture plates by 1000ml tip of micropipette and were filled

with 25, 50, 75µl of each plant extract. All the petriplates were kept in an incubator at 37°C for 24 hrs (not in an inverted position). After proper time of incubation growth of microbes was checked in all the Petri plates. After incubation for 24 hrs the plates were observed for zone of inhibition, the zone of inhibition was measured with scale and the observation was recorded on table.

Minimum Inhibitory Concentration (MIC)

To perform the MIC experiment we took six test tubes, washed and dried them. Poured 3 ml nutrient broth to each test tube and autoclaved them. 1ml plant extract was added to the first test tube, mixed it properly then 1ml mixture of this tube was added to the next (second) test tube. Likewise taken 1ml from second test tube and added it to the third test tube. Repeated the procedure till the sixth test tube. Discarded 1ml from the last test tube then 40 µl bacterial cultures were added to each test tube and incubated for overnight in shaker. Then after incubation taken optical density in spectrophotometer at 595nm.

Result and Discussion

Multiple Drug Resistance

Different chemical compounds present in the plant extract are mainly responsible for the antimicrobial activity. These compounds are diffused through the agar medium and depending on their concentration form the zone of inhibition (inhibition ring) and inhibit the growth of microorganism. Zone of inhibition can be known by measuring the diameter of inhibition ring in mm.

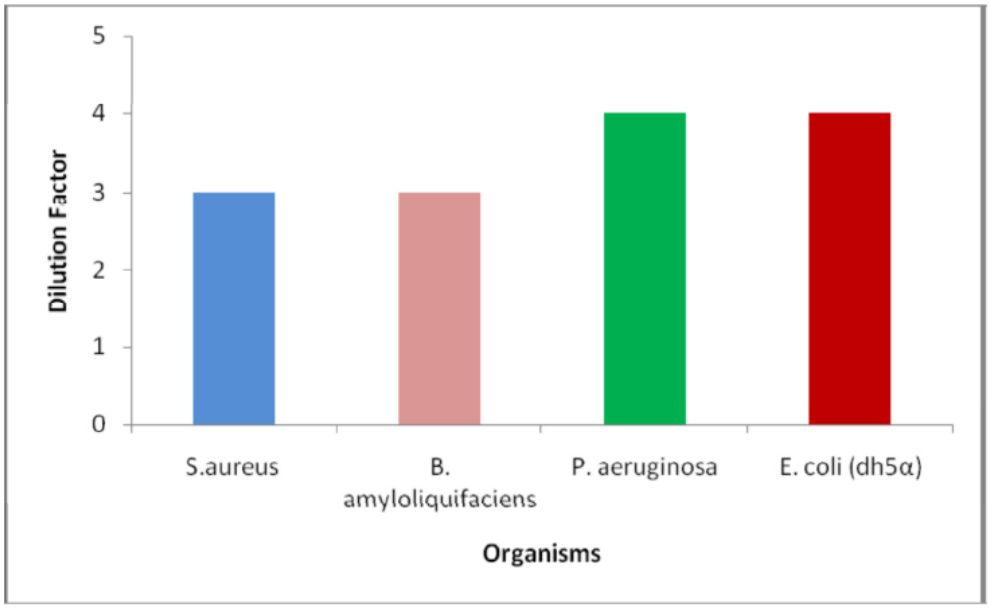
MDR with standard drugs

The results of zone of inhibition of sample ethanolic plant extracts Phyllanthus emblica) for four bacterial species Pseudomonas aeruginosa, Bacillus amiloliquifaciens, Staphylococcus aureus and Escherichia coli through standard antibiotics (tetracycline and chloramphenicol). Ethanolic extract of Phyllanthus emblica indicated maximum resistance for Pseudomonas aeruginosa and Escherichia coli.

Antibiotic Conc. (µg)	25				50				75			
Diameter of Zone of Inhibition in mm												
Antibiotic/Microorganism	S	B	P	E	S	B	P	E	S	B	P	E
Tetracycline	21	11	14	18	26	16	2	24	3	23	22	23
Chloramphenicol	32	-	-	32	34	-	15	36	36	-	25	38

Minimum Inhibitory Concentration (MIC)

It is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Agar diffusion techniques are used widely to assay plant extracts for antimicrobial activity. Factors affecting MIC are variation in incubation time, variation in temperature, variation in pH of broth etc.



Dried fruits of amla are used in the treatment of haemorrhage, diarrhoea and dysentery in Unani system of medicine. *officinalis* is also reported to have antiviral, anti-bacterial anti-fungal anti helminthic and anti-inflammatory properties24. Several of the bioactive compounds in *E. officinalis* such as flavonoids (quercetin), ascorbic acid, gallic acid, alkaloids (phyllantine, phyllantidine), hydrolysable tannins (emblicanin A and B), punigluconin and pedunculaginhave been identified. The antioxidant activity of *E. officinalis* has been attributed to the presence of tannins such as emblicannin A and emblicannin B13. In view of its medicinal and antimicrobial properties the present study aimed at assaying quantitatively the antibacterial activity of extracts of fruits of *E. officinalis* against *Staphylococcus aureus* and *E. coli* and thereby compare differentially the antibacterial action on gram positive and gram negative bacteria.

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