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Antimicrobial and antioxidant activity of leaf extracts of *Aegle marmelos*

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ABSTRACT

In the present investigation was undertaken to develop an Antimicrobial and Antioxidant activity of leaf extract of Aegle marmelos. From the leaf were extract with Acetone, Chloroform, Ethanol and Hexane against Bacterial strain like Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli and fungal strain like Aspergillus niger, A. flavus and Fusarium. The antimicrobial activity was determined by disc diffusion method. Acetone and hexane extracts were found to be highly active against bacterial species like Bacillus subtilis and Pseudomonas aurogenosa and fungal species like Aspergillus niger. The MIC values were obtained by serial dilution method. Ethanol fraction of leaves exhibited highest radicals scavenging activity, that is, 63.84 ± 0.05. In overall comparison the ethanolic extract of leaves in Aegle marmelos shows the highest scavenging activity followed by the acetone.

Key words: *Aegle marmelos, Bacillus subtilis, Aspergillus niger*, Antioxidant activity and Leaf extract.

INTRODUCTION

The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Today, as much as 80% of the world's population depends on traditional medicine as primary health care needs. With the recent advancement of research in the field, it has become apparent that many of the species utilized by indigenous people as well as the knowledge of traditional healers has begun to make its mark on society as a possible avenue for curing diseases. Recently, most of the research conducted in the traditional medicines has shown that some remedies obtained from traditional healers are very effective in spite of the fact that there is no scientific justification. The greater part of traditional therapy involves the use of plant extracts on their active principle (WHO, 1993).

Microorganism and medicinal plants are rich sources of secondary metabolites, which are potential sources of useful drugs and other useful bio reactive product. Scientific experiments on the antimicrobial properties of plant components were first documented in the 19th century. Microorganisms are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. Plants are used as medicines since time immemorial. Antibacterial properties of various plant parts like leaves, seeds, and fruits have been well documented for some of the medicinal plants for the past two decades. Antibiotic principles are the distributed widely among angiosperm plants. A variety of compounds is accumulated in plant parts accounting for their constitutive antimicrobial activities (Vlietinck, and Lindsay, 1995).

In recent years multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use and commercial antibacterial drugs commonly used in treatment and injections diseases. This situation forced scientists for searching new antimicrobial substances from various sources like medicinal plants which are the good sources and novel antimicrobial chemotherapeutic agents (Karaman *et al.*, 2003). The alarming increase in the rate of infection by antibiotic resistant microorganism has urged scientist to search for compounds which have potential antimicrobial activity (Dvis P. H. 1982). The ability to synthesis the compound by secondary metabolism possessing antimicrobial potential makes plants an invaluable source of pharmaceutical and therapeutic products (Lis-Balchin. M

and Deans S. G. 1997). The effectiveness of plant extracts on microorganism has been studied worldwide (Ates. D. A and Erdogrul. O. T. 2003).

Aegle marmelos has been known to be one of the most important medicinal plants of India. More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders (Badam. L *et al*., 2002). Antioxidant, antiulcer, antidiabetic, anticancer, antihyperlipidaemic, anti-inflammatory, antimicrobial and antispermatogenic effects have also been reported on various animal models by the crude extracts of this plant (Kamalakannan .P and Stanely. M. 2005). Every part of *Aegle marmelos* plant such as its fruits, stem, bark, and leaves possesses medicinal property and is used for treating various eye and skin infections. Leaf is considered to be one of the highest accumulatory parts of the plant containing bioactive compounds which are synthesized as secondary metabolites (Cowan. M. M. 1999). The present study was, therefore, aimed at evaluating the antimicrobial and antioxidant activity of *Aegle marmelos* leaf extracts against some pathogenic microbes.

MATERIALS AND METHODS

Leaf extraction

The leaves of *Aegle marmelos* were collected from in and around areas of Thanjavur, Tamil Nadu, India. The collected leaves were washed with running tap water and shade dried. After they were powder using a grinder. The powder was dissolved with acetone, ethanol, chloroform and hexane using soxhlet apparatus separately. The extracts were dried and dissolved in DMSO (Dimethyl sulfoxide) solution and screened for antimicrobial and antioxidant activity.

Antimicrobial activity

The antimicrobial activities was done by using bacteria strain like *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli* and fungal strain like *Aspergillus niger, A. flavus* and *Fusarium*. All the strains were collected from Microbiology laboratory, Medical college, Thanjavur. The antimicrobial activity was determined by disc diffusion method (Bauer *et al.*, 1966). Three different concentrations of 10mg/ml, 5mg/ml and 1mg/ml respectively were prepared. Each sterile disc was loaded with 100µl of test extract and placed on the agar plates inoculated with respective micro organisms. The plates were kept for half an hour for pre incubation diffusion. Then the plates were kept for incubation at 37°C for 24 hrs for bacteria and 48 hrs for fungi. At the end of incubation zones around the discs were measured. The study was performed in triplicate.

Determination of Minimum Inhibition Concentration

The minimum inhibitory concentration was determined by serial dilution method. Serial dilution of the extract was prepared in the test tubes containing peptone water as diluents. Fifty mg of the extract was dissolved in one ml of DMSO which is further subjected for two fold dilution. Totally 10 test tubes were maintained. The final concentration of the extract was now one half of the original concentration in each test tube. Each bacterial isolate was inoculated at 37°C for 24hrs. The tubes were then examined for the presence of growth considering turbidity as criterion. The highest dilution in each series that did not show turbidity and thus no growth was considered to be the MIC of the organism.

Determination of Antioxidant activity

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of Hatano (1988) and Bhuiyan (2009) with some modifications. The following concentrations of leaf extracts were prepared 40, 60, 100, 130 and 160 µg/ml, respectively. A stock solution of the sample (100 mg/ml) was diluted up to five concentrations. Each concentration was tested in triplicate samples. The portion of sample solution (0.5 ml) was mixed with 3.0 ml of 0.1 mM 1, 1-diphenyl-2-2picrylhydrazyl (DPPH, in 95% distilled ethanol) and allowed to stand at room temperature for 30min under light protection. The absorbance was measured at 518 nm spectrophotometrically. The scavenging activity of the samples at corresponded intensity of quenching DPPH lower the absorbance of the reaction mixture and indicates higher free radical scavenging activity. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the equation given below. In the DPPH test, antioxidants were typically characterized by their IC50 value (Inhibition Concentration of Sample required to scavenge 50% of DPPH radicals). The equation is: Scavenging effect (%) = (1-As/Ac) ×100 (As is the absorbance of the sample at t =0 min; Ac is the absorbance of the control at t=30 min).

RESULTS

Antimicrobial activity

The different extracts of *Aegle marmelos* tested for antibacterial activity and anti fungal activities on human pathogens were presented in Table 1 and 2. The zone of inhibition around the disc impregnated with plant extract over the lawn of bacterial and fungal culture plates determined the antimicrobial activity as quantitatively. The result showed that the antimicrobial activities of plant extract were increased with increasing concentration of crude extracts. The extracts were showed the prominent antimicrobial activity against with some pathogenic bacteria and fungus.

Antibacterial activity

The *Aegle marmelos* was found to be the highest zone of inhibition were observed in Acetone extract in 10mg/ml concentration was against *P. aeruginosa* and *Bacillus subtilis* (20mm) and Hexane extract also in 20mm at 10mg/ml concentration in each strain and lowest zone were observed against *K. Pneminia* (5mm) at 10mg/ml concentration in acetone extract. Acetone extract also showed good inhibitory activity against these strains and the zone of inhibition obtained were 20mm, 19mm, 16mm, 11mm and 5mm respectively (Table.1). Table 2 shows the MIC values obtained against *Bacillus subtilis* and *P. auerogensa*, which is same for both the strains (10.5mg/ml).

Antifungal activity

The *Aegle marmelos* was found to be more efficient in controlling the growth of *A. niger, with* the Zone of inhibition was observed in 19 mm in acetone and 17 mm in hexane at 10mg/ml concentration respectively (Table.1). The *Fusaricum* showing no inhibition zone against the all extracts.

Antioxidant activity

The yield of extracts using water and ethanol in case of *Aegle marmelos* was 1.45 and 1.90 g, respectively. The variation in yield may be due to the polarity of the solvents used in the extraction process. Table 3 shows the results of the free radical (DPPH) scavenging activity in (%) inhibition in *Aegle marmelos.* The result revealed that the ethanol fraction of leaves exhibited highest radicals scavenging activity, that is, 63.84 ± 0.05. The *Aegle marmelos* leaf extracts obtained from ethanol shows the value as 63.84 ± 0.05 which is the highest scavenging activity with 160 µg/ml of the crude extract followed by its water extract as 33.84 ± 0.05 with the same concentration. In overall comparison the ethanolic extract of leaves in *Aegle marmelos* shows the highest scavenging activity followed by the water.

Table.1. Antimicrobial activity of different extracts of *Aegle marmelos* against some pathogens

S.No	Test organism	Zone of Inhibition (mm)											
		Acetone			Chloroform			Ethanol			Hexane		
		10 mg/ml	5 mg/ml	1 mg/ml	10 mg/ml	5 mg/ml	1 mg/ml	10 mg/ml	5 mg/ml	1 mg/ml	10 mg/ml	5 mg/ml	1 mg/ml
1	*P.aeruginosa*	20	10	8	6	-	-	6	-	-	20	10	-
2	*B.subtilis*	20	15	11	6	-	-	16	10	-	20	11	6
3	*S.aureus*	16	12	10	-	-	-	15	12	9	-	-	-
4	*K.pnemonia*	5	-	-	5	-	-	11	-	-	-	-	-
5	*E. coli*	11	7	6	8	5	-	-	-	-	8	-	-
6	*A.niger*	19	11	7	5	-	-	-	-	-	17	10	-
7	*A.flavus*	-	-	-	-	-	-	14	7	6	10	-	-
8	*Fusaricum*	-	-	-	-	-	-	-	-	-	-	-	-

Table.2 Minimum inhibitory concentration (mg/ml) of Acetone and Ethanol extracts of *Aegle marmelos*

S.No	Extracts	Bacteria	
		B.subtilis	***S.aureus***
1	Acetone	10.5	10.5
2	Ethanol	10.5	10.5

Table.3 Specific antioxidant activity of different concentration of *in vitro* leaf extracts of *Aegle marmelos* with the Acetone and ethanol extracts

Sl. No	Concentrations of extracts (µg/ml)	Antioxidant activity	
		Acetone extracts	Ethanol extracts
1	40	25.3 ±0.02	53.0±0.04
2	60	27.6 ±0.08	56.9±0.06
3	100	30.0 ±0.10	60.0±0.09
4	130	32.3 ±0.07	61.5±0.07
5	160	33.8 ±0.05	63.8±0.05

DISCUSSION

In the present investigation revealed that the some of the traditional medicinal against with human pathogenic microorganism. Vlietinck, and Lindsay, (1995) reported that the Antibacterial properties of various plant parts like leaves, seeds, and fruits have been well documented for some of the medicinal plants for the past two decades. Antibiotic principles are the distributed widely among angiosperm plants. A variety of compounds is accumulated in plant parts accounting for their constitutive antimicrobial activities.

Prasannabalaji *et al.*, (2012) investigated that the newer antibacterial bioactive compounds targetted on the unexplored folk medicinal plants, being used for centuries in treating local population. The plant extracts are considered as best source of bioactive compounds particularly for traditional healers as they contain components of therapeutic values. The bioactive compounds have been detected for either bacteriostatic or bacteriocidal property and have very minimum or no toxicity to host. The *Aegle marmelos* was found to be the highest zone of inhibition were observed in Acetone extract in 10mg/ml concentration was against *P. aeruginosa* and *Bacillus subtilis* (20mm) and Hexane extract also in 20mm at 10mg/ml concentration in each strain and lowest zone were observed against *K. Pneminia* (5mm) at 10mg/ml concentration in acetone extract.

Table 2 shows the MIC values obtained against *Bacillus subtilis* and *P. auerogensa*, which is same for both the strains (12.5mg/ml). A similar type of study made with leaf extract of *Morinda citrifolia L.* (Usha *et al* 2010). Showed that ethanol extract of was highly effective against *Staphylococcus aureus* and *Bacillus subtilis* apart from other strains. Similarly the acetone extract of *Cassia auriculata* flower also inhibited the growth of these two strains (Maneemegalai and Naveen 2010). However, the extract here was found to be a broad spectrum microbial inhibitor. The present study indicates that the phytochemicals of *Aegle marmelos* has significant inhibition for *Bacillus subtilis* and *P. auerogensa.*

Meenu sharma and Suman joshi (2011) reported that the active anti-oxidant compounds are better extracted in methanol for *Aegle marmelos* and in ethanol for *Tinospora cordifolia.* Results also suggest that there is a direct co-relation between the total polyphenols extracted and anti-oxidant activity. In the present study reported that the ethanol fraction of leaves exhibited highest radicals scavenging activity, that is, 63.84 ± 0.05. In overall comparisons of ethanolic extract leaves in *Aegle marmelos* shows the highest scavenging activity followed by the water (Table.3).

CONCLUSION

The present study concluded that the good antimicrobial and antioxidant activity of different leaf extracts of *Aegle marmelos* were against some pathogenic microbes. Further work need to be done on these extract to isolate active component and treatment of infectious diseases.

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