ANTI-OXIDANT AND ANTI-MICROBIAL ACTIVITY OF LEAF EXTRACT OF BAEL LEAVES (AEGLEMARMELOS)

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Abstract

In the present examination was attempted to build up an Antimicrobial and Antioxidant activity of leaf extract of Aeglemarmelos. From the leaf were extricate with Acetone, Chloroform, Ethanol and n-Hexane against Bacterial strain like Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli and parasitic strain like As per gillusniger, A. flavus and Fusarium. The antimicrobial activity was determineddisc diffusion strategy. Acetone and hexane extricates were observed to be found to beenergetic against bacterial species like Bacillus subtilis and Pseudomonas aurogenosa and fungal species like Aspergillusniger. The MIC esteems were acquired by sequential dilutions technique. Ethanol portion of leaves displayed most amazing radicals scavenging activity that is, 64.60 ± 0.05. In by and overall comparison the ethanolic concentrate of leaves in Aeglemarmelos showed the maximum scavenging activity followed by the acetone.

Key Words*: -Aeglemarmelos (Bael), Bacillus subtilis, Aspergillusniger, antimicrobial, antioxidant and leaf extracts.*

Introduction

Aeglemarmelos belonging to family Rutaceae, is commonly known as Bael in indigenous systems of medicine and has been regarded to possess various medicinal properties. Bael is indigenous to India and its leaves, bark, roots and fruits have been used for olden days in the Indian traditionalsystem of medicine, plant extracts been used by the village people in folk medicine to treat various diseases. The infusion of dried unripe fruits has been used antidiarrheal and antidysensetry agents, the juice from crushed leave has been used for the treatment of bronchitis, and the decoction of root barks has been as anti-malaria drug.Clinical studies prove that bael possesses anti-diabetic, diuretic, antifertility, anticancer, chemo preventive, ulcer healing, antifertility and anti-inflammatory properties, which help it to be useful in prevention and treatment of many diseases. Microorganism and medicinal plants are rich sources of secondary metabolites, which potential sources of useful drugs and other useful bio reactive product. In recent years drug resistance in human pathogenic microorganisms has developed due to indiscriminate use and commercial antibacterial drugs commonly used in treatment and injections diseases.

MATERIALS AND METHODS

Leaf extraction

The leaves of Aeglemarmelos were gathered from in and around regions of Visakhapatnam and Srikakulam Districts, India. The gathered leaves were washed with running faucet water and shade dried. After they were powder utilizing a processor. The powder was dissolved with acetone, ethanol, chloroform and hexane utilizing soxhlet apparatus. The concentrates were dried and dissolved in DMSO (Dimethyl sulfoxide) solution and screened for antimicrobial 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 Aspergillusniger, A. flavus and Fusarium. All the strains were collected from Microbiology laboratory, Molecular Biology division, MicGene Lab, Visakhapatnam-17, Andhra Pradesh. India. 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 microorganisms. The plates were kept for half an hour for pre incubation diffusion. Then the plates were kept for incubation at 37°C for 24hrs for bacteria and 48hrs 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. Took 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 different 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 or 1.0ml) was mixed with 2.0 ml of 0.1 mM 1, 1-diphenyl-2-2picrylhydrazyl (DPPH, in 90% distilled ethanol) and allowed to stand at room temperature for 1hr 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) were 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= 1 hr).

RESULTS

Antimicrobial activity

The different Concentration of *Aeglemarmelos* tested for antibacterial activity and anti-fungal activities on human pathogens were presented in Table 1 and Table 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 *Aeglemarmelos* 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 *Aeglemarmelos* 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 *Aeglemarmelos* 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 *Aeglemarmelos.* The *Aeglemarmelos* leaf extracts obtained from ethanol shows the value as 64.60 ± 0.05 which is the highest scavenging activity with 160 µg/ml of the crude extract followed by its water extract as 34.60 ± 0.05 with the same concentration. Overall comparison the ethanolic extract of leaves in *Aeglemarmelos* showed the highest scavenging activity.

Table.1. Antimicrobial activity of different extracts of *Aeglemarmelos* against pathogens

S.No	Test organism	Zone of Inhibition (mm)											
		Aceto ne			Chlorof orm			Ethan ol			Hexa ne		
		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*	21	9	8	7	-	-	7	-	-	21	9	-
2	*B.subtilis*	19	16	11	5	-	-	15	12	-	19	12	6
3	*S.aureus*	16	13	10	-	-	-	16	10	8	-	-	-
4	*K.pnemonia*	6	-	-	5	-	-	10	-	-	-	-	-
5	*E. coli*	10	6	6	9	5	-	-	-	-	7	-	-
6	*A.niger*	20	11	7	4	-	-	-	-	-	87	10	-

7	*A.flavus*	-	-	-	-	-	-	14	7	7	10	-	-
8	*Fusaricum*	-	-	-	-	-	-	-	-	-	-	-	-

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

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

Table.3 Antioxidant activity of different concentration of *in vitro* leaf extracts of *Aeglemarmelos* with the Acetone and ethanol extracts

Sl. No	Concentrations of extracts (µg/ml)	Antioxidant activity	
		Acetone extracts	Ethanol extracts
1	40	24.2 ±0.02	52.0±0.04
2	60	28.4 ±0.08	55.0±0.06
3	100	29.0 ±0.10	61.0±0.09
4	130	33.0 ±0.07	60.5±0.07
5	160	34.6 ±0.05	64.6±0.05

DISCUSSION

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. A variety of compounds is accumulated in plant parts accounting for their constitutive antimicrobial activities. 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 *Aeglemarmelos* 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.

Ethanol extract of was highly effective against *Staphylococcus aureus* and *Bacillus subtilis* apart from other strains. However, the extract here was found to be a broad spectrum microbial inhibitor. The study indicates that the phytochemicals of *Aeglemarmelos* has significant inhibition for *Bacillus subtilis* and *P. auerogensa.*The active anti-oxidant compounds are better extracted in methanol for *Aeglemarmelos* and in ethanol for *Tinosporacordifolia.* 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, 64.60 ± 0.05. In overall comparisons of ethanolic extract leaves in *Aeglemarmelos* shows the highest scavenging activity followed by the water (Table.3).

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