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Research Article



Antimicrobial activity of *Aegle marmelos*

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Abstract

The Present study of medicinal plants as antimicrobial agents is necessary for gaining insight into medicinal flora and their real value. In this study, crude extract of *Aegle marmelos* showed maximum antibacterial activity against *Staphylococcus aureus* and its MBC was found to be 7.8 mg/ml. All other bacterial species showed moderate activities and the MBC were between 7.8 – 62.5 mg/ml. The crude extract also exhibited maximum antifungal activity against *C. albicans* and its MFC was found to be 15.6 mg/ml. All other fungal species showed moderate activities and their MFC were between 31.2 – 62.5 mg/ml. The results revealed that the crude extract of *A. marmelos* is bacteriostatic at lower concentration but cidal at higher concentration, in most of the organisms studied, probably due to the interference by the active principles of the extract. The presence of allylphenol like allylpyro catechol monoacetate, chavibetol acetate and eugenol could be correlated to the antibacterial and antifungal activity of the *A. marmelos* crude extract. The results of the present study provide a scientific validation for the popular use of *A.marmelos*. However further phytochemical work on the isolation and identification of the active compounds with antibacterial activity and antifungal activity is warranted. Further investigations must be performed to examine the antifungal properties to other pathogenic fungi and bacteria at a higher concentration. It is quite evident from this review that *Aegle marmelos* contains a number of phytoconstituents which reveals its uses for various therapeutic purposes. The Plant or its individual parts can be used for the treatment of various disorders in human being such as, diabetes, liver toxicity, fungal infection, microbial infection, inflammation, pyrexia and to relieve pain. Still, so much work is required with the *Aegle marmelos* to investigate the mechanism of actions with other therapeutic activities.

Keywords: *Aegle marmelos*, Antimicrobial activity and Phytochemical analysis.

Introduction

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Robin et al., 1998; Davis, 1994; Mulligen et al., 1993). However, the situation is a laming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients

(Rinaldi, 1991; Diamond, 1993). In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of either to unknown disease causing microbes, pose enormous public health concerns (Maurice, 1991).

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today plant materials continue to play a major

role in primary healthcare as therapeutic remedies in many developing countries (Zakaria, M. 1991). The different systems of medicine practiced in India, Ayurveda, Siddha, Unani, Amchi and local health traditions, Utilize a large number of plants for the treatment of human diseases. Most of these medicinal plants have been identified and their uses are well documented and described by different authors (Saradamma, 1990; Kirtikar, et al; 1991).

Scope and plan of work

Infectious diseases account for a larger proportion of health problems in the developing countries and the use of steroids, antibiotics and cytotoxic drugs has increased the absolute number of microbial infections. Since antimicrobial activity of plants and synthetic compounds remains largely unexplored, interest has grown in the exploration of antimicrobial activity from plant sources and synthetic compounds. Though the discovery of bacterial agents such as tetracycline, erythromycin and penicillin are revolutionized the therapy of microbial infections, these antimicrobial agents show varying degree of toxicity ranging from vomiting to hepatic and nephritic failure. Further these antimicrobial agents are very expensive.

Hence there is a need for new antimicrobial compounds with broad spectrum activity, which are cheaper and with less toxicity. Household medicinal plant may have some antimicrobial activity which will be not only cheaper but exert less toxicity since they are already in use. Many researchers worked on the search and evaluation of novel compounds. In this study we have tested the leaf extracts of *Aegle marmelos* for antimicrobial activity.

Antimicrobial activity of these extracts and compounds were studied against seven bacterial species and seven fungal species. The bacterial species include Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus vulgaris* and *Vibrio cholerae*).

Materials and Methods

Microorganisms

Antimicrobial activity of plant extracts and synthetic compounds were investigated on seven bacterial

species and seven fungal species. The bacterial strains include one Gram positive bacterium *Staphylococcus aureus* and six Gram negative bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhimurium* and *Vibrio cholerae*). The fungal species used in this study are *Aspergillus niger*, *A. fumigatus*, *A.flavus*, *Mucor* species, *Rhizopus* species, *Penicillium* species and *Candida albicans*. The microorganisms and chemicals were obtained from Hubert Enviro care systems (P) Ltd, Chennai, Tamilnadu, India.

Cleaning of glassware's

All glassware's were kept in chromic acid cleaning solution (10% potassium dichromate in 25% sulphuric acid) for a few hours. The glassware's were washed thoroughly in tap water, followed by detergent solution and finally rinsed with distilled water, and then they were dried in dust-proof cupboard.

Sterilization

Media were sterilized in an autoclave at 15 Lbs pressure for 20 min. The glassware's were sterilized in a hot air oven at 121⁰c for 3 hrs.

BACTERIAL MEDIUM PREPARATION

Muller-Hinton agar

Eight grams of Muller-Hinton agar was suspended in 1000ml of distilled water and the pH was adjusted to 7.3 and the agar was boiled to dissolve the medium was sterilized by autoclaving at 121⁰c (15Lbs for 15 minutes and mixed well before pouring.

Muller-Hinton broth

Twenty one gram of the Muller –Hinton broth powder was suspended in 1000ml and the PH was adjusted to 7.4 and the broth was boiled to dissolve the medium completely. The broth was sterilized by autoclaving at 121⁰c (15Lbs) for minutes.

FUNGAL MEDIUM PREPARATION

Sabouraud dextrose agar and Sabouraud dextrose broth

Peptone agar and dextrose were dissolved in water, boiled and stirred well. Chloramphenicol in 2ml ethanol [95%] was added to the hot medium.

Cycloheximide was dissolved in 2ml acetone and added, while stirring to the hot medium. Streptopenicillin was mixed with the hot medium was yellow and the final PH of the medium was 5.6 ± 0.2 .

Plant material

Aegle marmelos

The *Aegle marmelos* plant leaves were collected from in and around Vandhavasi. The taxonomic identity of the plants was established by the department of Life sciences, Madras University. Voucher specimens were maintained in the herbarium, Department of Botany.

Extract preparation

The plant materials were washed with tap water and then with sterile water. They were then macerated using mortar and pestle using sterile double distilled water at a concentration of one gram of tissue per milli litre on water [1:1 w/v] and filtered through gauze and the filtrates were evaporated at 45c. The concentrated extracts were weighed and dissolved in 5% dimethylsulfoxide [DMSO] individually.

IN VITRO SUSCEPTIBILITY TESTING FOR BACTERIAL SPECIES

One gram-positive bacterium [*Staphylococcus aureus*] and six Gram-negative bacterium [*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhimurium*, *Vibrio cholerae*] were used for the study.

Inoculum preparation

Inoculums of each bacterial strain was suspended in 5ml of Muller-Hinton broth and incubated overnight. Cultures were diluted 1/10 with Muller-Hinton broth before use.

Disc-Diffusion method [Bauer et al., 1996] Preparation of disc

Whatman No.1, 6mm filtered paper disc were prepared and sterilized by autoclaving. These discs were plated and each disc was impregnated with appropriate quantity of stock and dried overnight at 31⁰c. This was carried out under sterile condition inside a laminar flow.

Inoculation and testing

Each Muller-Hinton agar plates were inoculated with the standard inoculum suspension by soaking a swab and rotating it over the agar plate. The paper disc was placed over the inoculated agar. After 24 hrs of incubation at 37⁰c, zone of inhibition of growth was measured and recorded. Three replica plates were maintained.

TUBE DILUTION METHOD

From the plant extract [250mg/ml,] 0.5ml was incorporated into 0.5ml of Muller-Hinton broth to get a concentration of 125mg/ml and serially diluted by double dilution technique to achieve 62.5mg/ml, 31.2mg/ml, 15.6mg/ml, 7.8mg/ml, 3.9mg/ml, respectively [Sham et al.,1991].

Minimum inhibitory concentration [MIC]

For MIC determination 0.5ml of various concentrations of extract [125 to 1.95mg/ml] and synthetic compounds [50 to 0.78ul] of bacterial strains inoculum was transferred on to each tube. The last tube of Muller-Hinton broth with 50 µl of inoculum served as positive control. The whole set up in triplicate was incubated at 37⁰ c for 24 hrs. The MIC was the lowest concentration of the extract that did not permit any visible growth after 24 hrs incubation.

Minimum bactericidal concentration [MIC]

The MBC was determined by sub culturing the above [MIC] serial dilutions after 24 hrs, in Muller-Hinton agar plates using 0.01 µl loop and incubating at 37⁰ c for 24 hrs. MBC was regarded

Results and Discussion

The antimicrobial efficacy of plant materials was well documented. Although the nature and number of active antimicrobial principles involved in each extract are not clear, but the broad spectrum activity of plant extracts (*A. marmelos*), however is promising. Two of the least susceptible bacteria were *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*.

The latter is the prevalent burn-patients pathogen capable of causing life-threatening illnesses. Some strains causing septicemia and pneumonia in cystic fibrosis and immunocompromised patients are

becoming difficult to treat with currently available antimicrobial agents. Due to multi-resistance of *Pseudomonas fluorescens*, there is a lack of active antibiotics effective against this bacterium, resulting in an increase in nosocomial infections and high mortality. It is also an opportunist associated with respiratory and urinary tract, wounds and bacteremia. Table 1 shows the zones of inhibition of plant extracts bacterial species. Water extracts of plants at a concentration mg/ml show varying zones of inhibition ranging from 22-26 mm diameter. Among the test species, *A. marmelos* crude extract showed higher zone of inhibition on *S. aureus*.

MIC and MBC of crude extract of *A. marmelos* against bacterial species are presented in table 3. MIC and MBC of *A. marmelos* crude extract are 3.9 mg/ml and 7.8 mg/ml respectively against *S. aureus*. In all other bacterial species MIC and MBC values range from 7.8-31.2 mg/ml and 15.6-62.5 mg/ml respectively. Both *P.aeruginosa* and *Vibrio cholerae* showed no activity up to 125 mg/ml.

Antibacterial activity of *A. marmelos* crude extract

The aqueous extracts of *A.marmelos* have been shown significant activity against *S.aureus*. The leaf extract showed antibacterial activity against *Cornybacterium diptheriae*, *Pneumococcus*, *Streptococcus pyrogens*, *E.coli*, *Salmonella typhimurium*, *Proteus vulgaris*, *Staphylococcus aureus* and *Klebsiella pneumoniae* [Bhatnagar et.,1961]

A total of 7 bacterial species consisting of one gram-positive and six Gram-negative bacteria were tested for their susceptibility towards *Aegle marmelos* extract. The degree of susceptibility varied depending on the species and when compared, Gram-positive bacterium *S. aureus* showed maximum susceptibility than Gram-negative bacteria.

S.aureus is a major pathogen for humans and almost every person will have some type of *S.aureus* infections during a life time, ranging in severity from food poisoning or minor skin infections to severe life treating infections. Therefore, the discoveries of these potential herbal antibacterial agents are encouraging in replacing the current commercial antibacterial drugs that induce many types of toxicities in patients.

The *A. marmelos* crude extract showed low

susceptibility against *P.vulgaris*. *P.aeruginosa* and *V.cholerae* showed no activity up to 125 mg/ml. The Acetogenins are a new class of natural compounds whose potent biological activity and special structures have reported some workers have isolated flavonoids from leaves [Seetharaman TR et al.,1986],aporphine alkaloids [Bhaumik et al., 1979; Bhakuni 1972], terpine derivatives [Bohlmann,1973],glycoside [forage et al 1980] and a novel diazepine, squamolone [yang TH,1972]were isolated from this plant. Numerous acetogenins have been shown to possess cytotoxic pesticidal, antimalarial, cell growth inhibitor, and inhibitory, antiparasitic and antimicrobial activities. Bullatacin is one such compound that possessed anti-tumoural and pesticidal activity in vivo. Methanolic extracts of *Aegle marmelos* seeds have been shown to have antiparasitic activity.

Table 3 shows the zones of inhibition of *A. marmelos* plant extracts against fungal species. Water extracts of plants at a concentration mg/ml showed varying zones of inhibition ranging from 8-16 mm diameter

MIC and MFC of crude extract of *A. marmelos* against fungal species are presented in table 4. MIC and MFC of *A. marmelos* crude extract are 15.6 mg/ml and 31.2mg/ml of respectively against *C .albicans*. In all other fungal species, the MIC and MFC values range from 31.2 mg/ml-62.5mg/ml and 62.5 mg/ml-125 mg/ml of respectively. *Mucor* species showed no activity up to 125 mg/ml.

Antifungal activity of *A. marmelos*

The results obtained from the present study suggested that *A.marmelos* plant extracts possess significant antibacterial property. *A.marmelos* crude extract exerted a strong antifungal activity against *C.albicans* are often implicated in the infections of genitourinary tract;consequently the reputed usefulness of extracts in treating venereal diseases might be due to their inhibitory effect against this group of fungal species [Dorothy et al.,1991]

The *A. marmelos* crude extract in our work also exhibited moderate antifungal activity against *A. fumigatus*, *A.niger*, *A.flavus*, *Rhizopus* species and *Penicillium* species. The *Mucor* species showed no activity up to 125 mg/ml. A previous investigation revealed that water extract from *A.marmelos* leaves contained potential antifungal agent against *Candidia*

Table – 1 Zones of inhibition of *Aegle marmelos* extracts

S.No	Name of the bacterial species	Zone of inhibition (mm)*
1	<i>Staphylococcal aureus</i>	26
2	<i>Klebsiella pneumoniae</i>	22
3	<i>Pseudomonas aeruginosa</i>	NA
4	<i>Proteus vulgaris</i>	22
5	<i>Escherichia coli</i>	24
6	<i>Salmonella typhimurium</i>	25
7	<i>Vibrio cholera</i>	NA

*-mean of three assays
NA –no activity

Table – 2 MIC and MBC of *Aegle marmelos* crude extract

Bacterial species	MIC(mg/ ml)	MBC(mg/ ml)
1. <i>Staphylococcus aureus</i>	3.9	7.8
2. <i>Klebsiella pneumonia</i>	15.6	31.2
3. <i>Pseudomonas aeruginosa</i>	NA	NA
4. <i>Proteus vulgaris</i>	31.2	62.5
5. <i>Escherichia coli</i>	7.8	15.6
6. <i>Salmonella typhimurium</i>	7.8	15.6
7. <i>Vibrio cholera</i>	NA	NA

MIC-minimum inhibitory concentration
MBC-minimum bacterial concentration

Table – 3 Antifungal activity of *A. marmelos* crude extract

S.No	Name of the fungal species	Zone of inhibitions (mm)*
1	<i>Candida albicans</i>	16
2	<i>Aspergillus niger</i>	9
3	<i>Aspergillus flavus</i>	11
4	<i>Aspergillus fumigates</i>	13
5	<i>Mucor species</i>	NA
6	<i>Rhizopus species</i>	13
7	<i>Penicillium species</i>	8

*-mean of three assays
NA-no activity

Table - 4 MIC and MFC of *Aegle marmelos* crude extract against fungal species

S.No	Name of the fungal species	MIC (mg/ml)	MFC (mg/ml)
1	<i>Candida albicans</i>	15.6	31.2
2	<i>Aspergillus niger</i>	62.5	125
3	<i>Aspergillus flavus</i>	31.2	62.5
4	<i>Aspergillus fumigatus</i>	31.2	62.5
5	<i>Mucor species</i>	NA	NA
6	<i>Rhizopus species</i>	31.2	62.5
7	<i>Penicillium species</i>	62.5	125

MIC- minimum inhibitory concentration

MFC- minimum fungicidal concentration

NA- no activity

albicans and antibacterial agent against *Escherichia coli* for the treatment of opportunistic infections in patients afflicted with acquired Immunodeficiency syndrome [AIDS]. These results were comparable to commercial antifungal drug Amphotericin B and antibiotic Chloramphenicol [Crockett et al., 1992].

As the toxicity studies reveal that this plant extract and their components does not produce any toxicity, the *A.marmelos* extract of and their active components can well be exploited for antifungal treatment. As fungal infections being a major cause of morbidity and mortality in immunosuppressed patients, this herbal extracts may prevent infections.

Further study on the fractionation of these plant extract machinery on infecting microbial species may provide a better understanding of the infection management.

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