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Research Article

Phytochemical Screening and Potential *In vitro* Anti-Bacterial Activity of *Aegle marmelos* Leaf Extract Against Gram Negative Pathogenic Bacteria.

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ABSTRACT

To investigate the phytochemical and antibacterial potential of leaves of *A. marmelos* against isolated human pathogenic gram negative bacterial strains from four different hospital isolates. Petroleum ether, Chloroform, Methanol and Aqueous extracts at three different concentrations (50, 75 and 100 mg/mL) were evaluated. Agar diffusion method was followed to evaluate the antibacterial efficacy. The phytochemical study revealed the presence of Alkaloids, Carbohydrates, Amino acids, Steroids, Flavonoids, Phenols and tannins in all four extracts but Saponins, Gum and Mucilage are absent in Petroleum ether and Chloroform extracts and they are present in Methanol and Aqueous extracts. All extracts of the leaf demonstrated significant antibacterial activity against tested pathogens. Among all extracts, ethanol extract has revealed the highest inhibition rate comparatively. The present study also favored the traditional uses reported earlier. Results of this Phytochemical and antimicrobial studies strongly confirm that the leaf extracts of *A. marmelos* have some primary and secondary metabolites and they could be effective antibiotics, in controlling gram-negative human pathogenic infections.

Key words: *Aegle marmelos*, Gram negative Bacteria, Phytochemicals

INTRODUCTION

The use of plants for medicinal purposes dates back to antiquity[4] and has been very important in the health care delivery of every nation at one stage or another. Recent research has focused on natural plant product as alternatives to the existing drugs for disease remedy in developing countries[5]. Plant–derived medicines have been part of traditional health care in most parts of the world for thousands of years and there is increasing interest in them as sources of agents to fight microbial diseases[6 7 8] . In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This situation, the undesirable side effect of certain antibiotics, and the emergence of previously uncommon infections, has forced scientists to look for new antimicrobial substitutions from various sources such as medicinal plants[1]. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents[2 3]. Infectious disease still remains an important cause of morbidity and mortality in man, especially in developing countries. Today, in India, many resort to the use of locally made herbal preparations (infusion, decoction or concoction and tincture) as an alternative therapy for salmonella-infections. Thus, the study sought to evaluate the phytochemistry and synergistic antibacterial potential of *A. marmelos* herbal preparations from different plant parts used in south India for the traditional treatment of gram negative bacterial infection is undertaken. The present study therefore investigated and compared the antibacterial activities of various extracts of *A. marmelos* against four gram negative pathogenic bacterial strains.

MATERIALS AND METHODS

Plant material: The leaves of *A. marmelos* were collected around Gulbarga University campus in June 2012. The plant was identified by a taxonomist and a voucher sample was deposited in the Herbarium of Medicinal Plants of the Department of Botany Gulbarga University Gulbarga, Karnataka. The leaves of the specimen were washed with tap water followed by 70% alcohol, & shade dried.

Chemicals: Methanol, ethanol, ethyl acetate, Petroleum ether, Diethyl ether, H_2SO_4, Chloroform, HCl, KOH, hexane, silica Gel 60-120 mesh, Tween 80 Phosphate buffer saline, FCR Reagent, all the chemical, solvents and reagents used were analytical grade obtained from Hi media.

Preparation of Extract: The extraction procedure used for the isolation of crude drug from plants has been practiced

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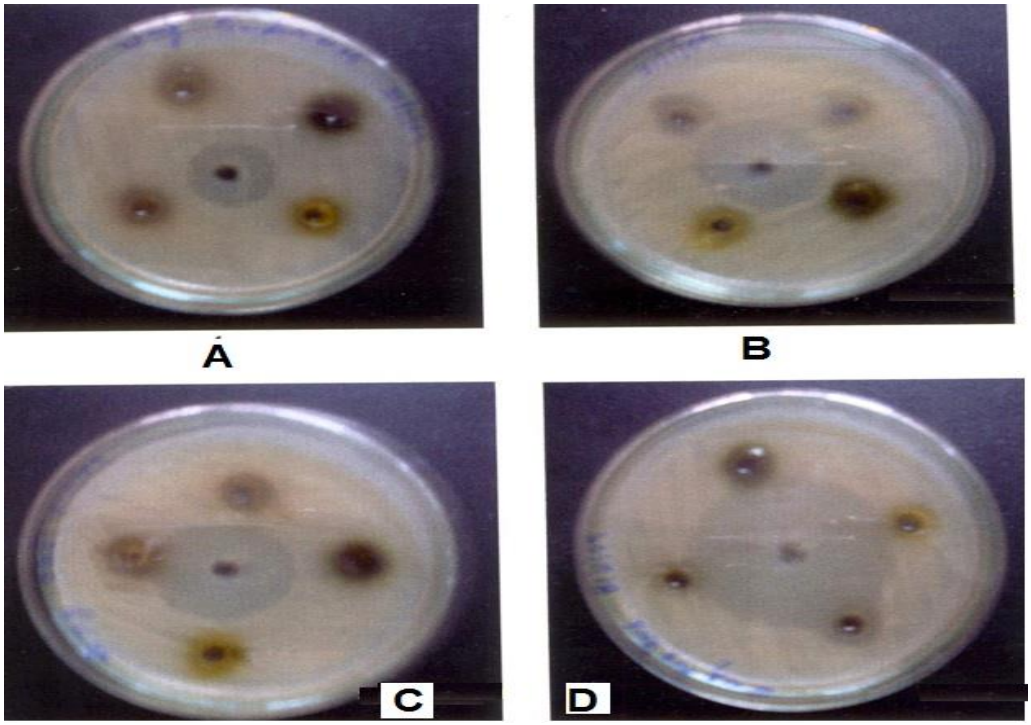

Fig 1: showing zone of inhibition
A: Staphylococcus aureus; B: Kleibsella spp; C: E.coli; D: Enterococcus spp

Table 1: Preliminary phytochemical analysis

	Alkaloids	Carbohydrates	Amino acids	Steriods	Saponins	flavonoids	Tannins	Gum and mucilage
Petroleum ether	-	+	+	+	+	-	-	-
Chloroform	+	+	+	+	-	-	-	-
Methanol	+	+	+	+	+	+	+	+
Aqueous	+	+	-	+	+	+	+	+

since long time. The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on type of substance that is being isolated. Plant material is shade dried before extraction. It is essential that drying operation is carried out under controlled condition to avoid chemical changes. It is essential for plant taken to be free from diseases i.e. not affected by bacterial or fungal infections. Normally the crude extract is taken by cold extraction with the help of non-polar to polar solvents. About 100 g of the shade dried powder leaves were taken separately and dissolved in 500ml of distilled water and magnetically stirred in a separate container for overnight at room temperature in the case of aqueous solution. Similarly 100gm of powdered leaves were dissolved in 500ml of different solvents such as Methanol, Chloroform, and Petroleum ether. These extract were then collected by evaporation in the plates and stored for further use.

Phytochemical studies of *A. marmelos:* The extracts obtained after each successive solvent extraction were qualitatively tested for the presence of various phytochemicals. The preliminary phytochemical screening was carried out.

Antibacterial susceptibility test

Microorganisms: The bacterial strains employed in this present study were collected from Dept. of Microbiology, Gulbarga University, Gulbarga Karnataka (India). This includes *Klebsiella pneumonia*, *E. coli*, *Staphylococcus aureus*, and *Enterococcus.* These species were originally isolated from clinical samples and identified by standard biochemical reactions.

Media: Nutrient broth (Hi Media M002) contain peptic digest of animal tissue (5g/L), yeast extract (1.50g/L), Beef extract (1.5g/L) was used for the growth of bacterial cultures. Antibiotic assay media No:11 (Hi Media MM004) congaing peptic digest of animal tissue (6g/L),

Casein enzyme hydrolyte (4g/L), Yeast extract (1.50g/L), Dextrose (1.00g/L), Agar (15.00g/L) was used for anti-bacterial activity.

Agar well diffusion method: The antibacterial activity of various extract of *A. marmelos* was determined by using agar well diffusion technique. For this 25 ml of sterile Muller-Hinton agar No.2 (Hi Media), was poured in sterile autoclaved Petri plates, before pouring 100µl activated bacterial culture was added, and then allowed to stand for solidification completely. The well was prepared with the help of sterile 6mm diameter cork-borer. Than 100µl of prepared crude extract (60mg/ml) solution were poured in to the wells. Then the plates were sealed with plasticize and transferred to refrigerator to diffuse out of 30 min. the plates were then

Table 2: Antibacterial activity of various extracts of *Aegle marmelos*

Bacteria	Zone of inhibition in mm												
	Petroleum ether mg/mL			Chloroform mg/mL			Ethanol mg/mL			Aqueous mg/mL			Streptomycin
	50	75	100	50	75	100	50	75	100	50	75	100	control
Sa	8	9	1	9	8	11	14	12	15	9	1	15	22
K spp	8	1	8	1	8	2	16	18	18	9	7	1	22
E.coli	7	9	8	11	1	1	15	1	13	7	12	9	22
E spp	12	11	9	1	1	2	21	13	18	1	6	9	20

incubated at 37ºC for 24 hrs. Triplicate plates were prepared for each treatment and the average zone of inhibition excluding well, were recorded. 0.01mg/mL Streptomycin was used as positive control. Inoculums turbidity was maintained constant throughout the experiment to 0.8 OD at 660nm. Level of turbidity is equivalent to approximately $1X10^{-8}$CFU/mL.

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) was determined through the broth dilution method. Bacteria were grown in Muller Hinton broth for 6 hrs. After this, 20µl of 106cells/mL were inoculated in tubes with Muller Hinton broth supplemented with 4 different concentrations (60, 40, 20, 10 and 5 µg/ml) of the various extracts. After 24 h incubation at 37 ºC the MIC of each sample was measured through optical density in the spectrophotometer at 660nm, though the non-inoculated Muller Hinton broth[9].

Statistical analysis: Results are expressed as Mean ± SD. The statistical analysis was carried out using one-way ANOVA analysis. The *p*-value of 0.05 or less was considered significant for all experiment.

RESULTS

Preliminary Phytochemical Test: In the present study preliminary phytochemical studies were carried out on leaves of *Aegle marmelos.* It was noticed that in some cases chemical constituents fail to answer due to trace amount or other reason. The extract obtained from successive solvents is subjected to phytochemical tests to reveal presence of different phytochemicals especially the primary and secondary metabolites present in the extract. However, the results are as shown in the table 1

Antibacterial activity of various extracts of *Aegle marmelos:* The results of the present study reveals that, the ethanol extract has shown maximum zone of inhibition against the all four pathogenic bacterial strains such as *Staphylococcus aureus, Kleibsella spp, E.coli, Enterococcus spp,* where in case of petroleum ether, chloroform and aqueous extracts which has shown moderate zone of inhibition against the same bacterial strains when compared to control. The diameter of zone of inhibition of various extract of *Aegle marmelos* against four pathogenic bacterial strains is shown in the fig 1 and also the results of the antibacterial activity evaluated by agar diffusion method are presented in the table 2.

Determination of MIC: The Minimum Inhibitory Concentration (MIC) of the crude drugs extracted from *Aegle marmelos* was tested at the concentration ranging from undiluted sample to 5mg/ml. the minimum inhibition concentration (MIC) for the crude drug for all tested gram negative bacterial strains was >5mg/mL.

DISCUSSION

Medicinal plants have played an important role in maintaining human health and improving the quality of human life since many years. The use of natural products for therapeutic purposes is in practice from ancient times. The world health organization has estimated that about 80% of the earth inhabitants relied on traditional medicine for their primary health care needs. Herbs have been used as food and medicine from many countries to cure the diseases. The traditional medicines are used to cure all kind of diseases.

The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant, such as alkaloids, flavonoids, steroids, tannins and phenolicterpenes, volatile oils which are synthesized and deposited in specific parts or in all parts of the plant. The plant secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential sites. Therefore, random screening of plants for bioactive chemicals is as important as, the screening of ethno botanically targeted species.

Phytochemical screening of *Cassia auriculata L* extract has revealed the presence of alkaloids, flavonoids, Phenolic, steroids and saponins in petroleum ether, chloroform and ethanol extracts[11], similarly in present study, the leaves of *Aegle marmelos* have been subjected to extract crude drug in 4 different solvents such as petroleum ether, chloroform, ethanol and aqueous by Soxhlet extraction method, the crude drug so obtained was tested for phytochemical studies which reveals the presence of phyto-constituents such as alkaloids, phenols, glycosides, flavonoids, tannins, gums and mucilayes etc.

Nowadays multiple drug resistance by the microbes is developed due to the indiscriminate use of drugs commonly used in the treatment of infectious disease treatment. Unfortunately, bacteria have the genetic ability to transmit and acquire resistance to drugs and chemicals[12]. The extra chromosomal genes associated with plasmids were found to be responsible for these antibacterial resistance phenotypes that may impart resistance to entire bacterial class[13].

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by microorganisms. In our study the four solvents extraction viz., petroleum ether,

chloroform, ethanol and aqueous extracts of plant *Aegle marmelos* have shown antibacterial activity against gram negative bacteria such has *Staphylococcus aureus, Kleibsella spp, E.coli, Enterococcus spp* which indicates the presence of broad spectrum of antibiotic compounds in the plant. This property will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are more prevalent in recent time. Different solvents have been reported to have the capacity to extract different phyto-constituents depending on their solubility or polarity and property of the solvent.

Therapeutic value of medicinal plants and bioactivity of extract lies in the various chemicals present in it, for instance, plant rich in tannins have antimicrobial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane[13]. Flavonoids are the major group of phenolic compounds reported to have antimicrobial activity[14]. The extracts of seeds of *Vitexagnus-castus*was reported to possess antimicrobial activity which is associated with its alkaloids, saponins, taninns, flavonoids and glycosides contents[15]. The antimicrobial activity of leaf extracts of *Aegle marmelos* as recorded in the present study may therefore be attributed to the presence of above phytoconstituents.

CONCLUSION

Based on the above observation, It is concluded that the plant extracts possess antibacterial activity against tested gram negative bacteria, the variation in the zone of inhibition suggesting that the varying degree of efficacy of different phyto-constituents of herb on the target organism. The antibacterial activity of the plants may be due to the presence of various active principles in their leaves. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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