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Antimicrobial effect of King of bitter *Andrographis paniculata* and traditional herb *Aegle marmelos* against clinical pathogens

S Rajeshkumar*

**Department of Biochemistry, Adhiparasakthi College of Arts and Science
Kalavai – 632506, Vellore District, TN, India**

Abstract: Plants are the most important source for all kind of food and medicine in India. From ancient time to modern world it is not possible to manufacture the medicine without plant or its phytochemicals. In India there are a lot of herbal plants have been used for the home medicine. In this present study Leaves extract of *Aegle marmelos* and king of bitter *Andrographis paniculata* are used for antimicrobial activity. The leaf extracts were prepared by using three solvents like aqueous, ethanol and acetone. All the three extracts were tested for antimicrobial activity against pathogenic micro-organisms especially *Staphylococcus aureus* and dermatophytes *Candida sp* using Agar well diffusion method. Among these three extracts ethanol extracts shows good antibacterial activity compared with aqueous and acetone extracts. It was observed that, ethanol extract of *Andrographis paniculata* showed the significant antibacterial activity against *Klebsiella pneumonia* and *candida sp* compared with *A. marmelos* extracts and aqueous and acetone extract of *A. paniculate.*
Key words: Antimicrobial activity, medicinal plants, *Aegle marmelos, Andrographis paniculate*.

Introduction

This medicinal herb *A. paniculata* otherwise called as king of bitters because it has extremely bitter in taste where it is used to treat various infections and diseases. The leaves and roots are highly used for medicinal purposes often being used before antibiotics were created[1]. It has blood purifying property so it is recommended for use in leprosy, gonorrhea, scabies, boils, skin eruptions, and chronic and seasonal fevers. Juice of fresh leaves used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection[2]. *A. paniculata* is having a number of bioactivities such as anti-inflammation, anti-cancer, immunomodulation, anti-infection, anti-hepatotoxicity, anti-atherosclerosis, anti-diabetes and anti-oxidation[3]. In this investigation we reported that phytochemical analysis of aqueous, acetone and ethanol extracts of commonly available, less expensive medicinal plant *A. paniculata* through chemical colour reaction and assessed its antimicrobial activity against pathogenic microorganisms.

Figure 1: The king of Bitter *Andrographis paniculata*

Aegle marmelos is the gods' tree, present in all lord Siva temples used from ancient days. *Aegle marmelos* is one of the traditional and a very important therapeutic plant throughout India (Figure 2). It is the family of Rutacae. *A. marmelos* is one of the imperative antibacterial agent against *Escherichia coli, Streptococcus pneumoniae, Salmonella typhi, Klebsiella pneumoniae* and *Proteus vulgaris, Staphylococcus aureus, B. subtilis, Pseudomonas aeruginosa, Proteus mirabilis, S. paratyphi* A, *S. paratyphi* B, *Micrococcus luteus, Enterococcus faecalis* and *Streptococcus faecalis*[4-8]. And also this plant has excellent antifungal activity against *Penicillium chrysogenum, Fusarium oxysporum, Trichophyton mentagrophytes, T. rubrum, Microsporum canis, M. gypseum, Epidermophyton floccosum, Aspergillus niger, Aspergillus fumigatus, Candida albicans* [4,6,9].

Figure 2: ***Aegle marmelos***

In this present investigation we identified the antimicrobial activity of the mainly important medicinal plants *A. paniculata* and *A. marmelos* against *Bacillus subtilis. Klebsiella pneumoniae, Klebsiella planticola, E. coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida sp*.

Experimental

Collection of plant leaves

All the culture media and analytical reagents were purchased from the Hi-media Laboratories Pvt, Ltd. Mumbai-India. The leaves were collected from Arcot, TN, India. The collected plant leaves were washed with running tape water and distilled water. Washed leaves were shade dried at room temperature for a week and grinded using mixer grinder and make into fine powder. After they are kept in air tight container and used to solvents extraction. Powdered leaves were subjected for extraction with aqueous, ethanol and Acetone using soxhlet apparatus.

Aqueous extraction

About 10 g of powdered leaves were mixed with 100 ml of sterile double distilled water and incubated on a water bath shaker for 12 h at 40°C. Then the mixture was filtered through Whatman No 1 filter paper, then the supernatant was collected and used for preliminary phytochemical analysis.

Acetone and acetone extraction

A 25gm of powdered leaves were extracted withorganic solvents by using Soxhlet apparatus. These were successively extracted with 80 % ethanol at 60°C for 48 h and 70%acetoneat 55°C for 48 h. The obtained extract was further filtered with Whatman No 1 filter paper and then allowed to evaporation. After evaporation, the sample was in the form of powder (concentrated form) and this form was stored at 4°C untilfurther use. During assay the bioactive compound was diluted by using double distilled water

Antimicrobial activity assay

The extract of *A. paniculata* and *A. marmelos* was tested for antimicrobial activity by agar well diffusion method against pathogenic Gram positive and negative bacteria are *Bacillus subtilis. Klebsiella pneumoniae, Klebsiella planticola, E. coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida sp.* Different volumes of crude plant extracts were dissolved in distilled water (10 mg/ml). Muller Hinton Agar medium was used to cultivate bacteria. Fresh overnight culture of each strain was swabbed uniformly onto the individuals plates using sterile cotton swabs. 6 wells were made on each Muller Hinton Agar plates with 5 mm

in diameter. Then the dilute extracts with different concentrations (25, 50 and 75 µL) were poured into each well on all plates. The commercial drug Ciprofloxacin was maintained as control and incubates for 24 h at 37°C. After incubation the different levels of zonation formed around the well was measured.

Result and Discussion

Antibacterial activity of aqueous, ethanol and acetoneextract

An aqueous extract of *A. paniculata* showed potent antibacterial activity against *Klebsiella pneumonia* (19 mm), *Bacillus subtilis* (18 mm) and *P. Aeruginosa* (17 mm) at 75µL concentration. On other hand aqueous extract showed moderate activity against dermatophyte (*Candida sp*), *S. aureus, K. planticola* and *E. coli.* Minimum inhibitory concentration of aqueous extract is 50 µL (Figure 3).

Similarly, acetone and ethanol extract of *A. paniculata* exhibited moderate degree of bacterial activity against all the seven tested bacteria (Figure 3).The acetone extract showed moderate bacterial activity than the water and ethanol extract. Among of the seven microbial strains investigated *Escherichia coli, S. aureus* and *Candida* sp. The standard drug Ciprofloxacin showed high degree of inhibition against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Klebsiella pneumoniae.*

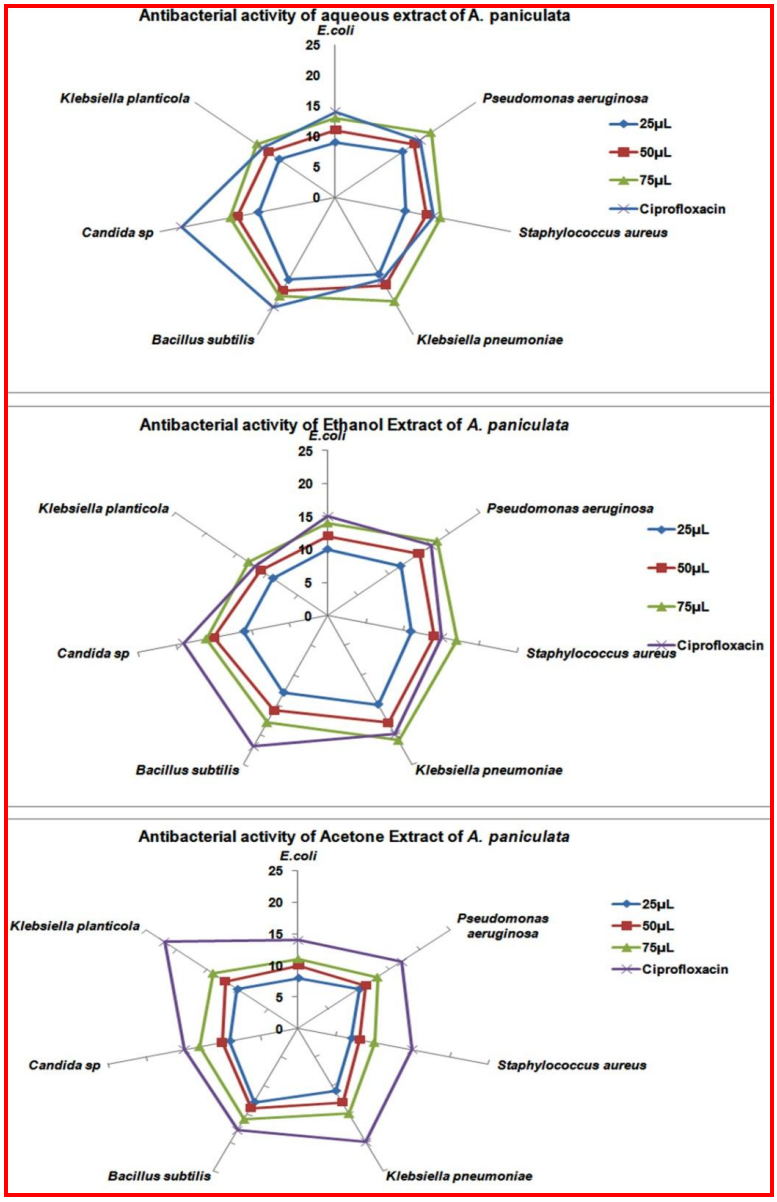

Figure 3 Antibacterial activity of aqueous, Ethanol and Acetone extract of *A. paniculata* against pathogenic bacteria

The antibacterial activity of *Andrographis paniculata* alcoholic extract tested against seven bacterial strains by MIC method was used in this investigation. The result of screening the antifungal activity of ethanolic extract *A. paniculata*by MIC was displayed in table 3. The ethanolic extract of leaves *A. paniculata* exhibited significant antimicrobial activity against dermatophytes of at the concentration of 50 µL and above. An ethanol extracts of *A. Paniculata* leaves showed effective antibacterial activity against *K. pneumonia*, *P. aueruginosa* (21 mm) and *S. aureus* (17 mm) at concentration of 75 µL and also show moderate activity against *K. planticola*, and *E. coli*. The development of zone is mainly based on the concentration of extract [10-12].

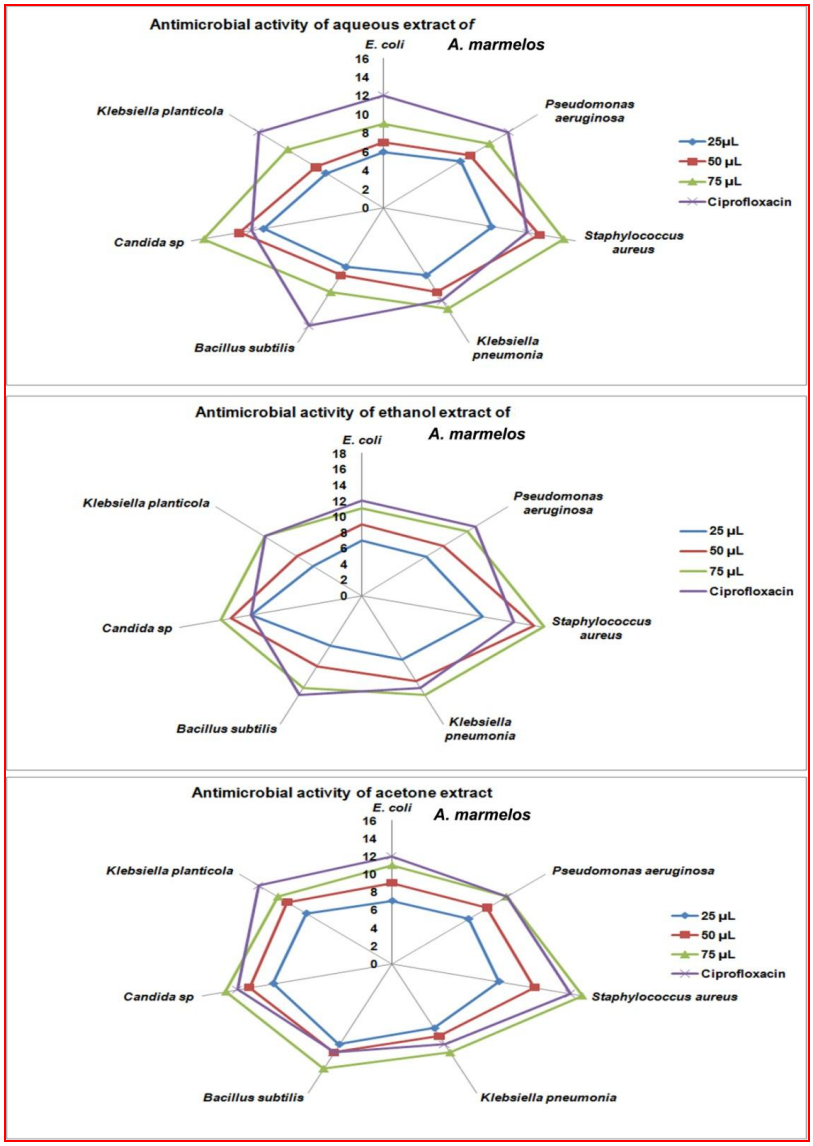

Figure 4: Antimicrobial activity of *A. marmelos*

The presence of phytochemicals will determine antimicrobial functions.[13-17]. When compare to *A. paniculata*, *A. marmelos* shows the lower amount of antimicrobial capability. In this all the extracts of *A. marmelos* were shown significant inhibitory activity on all strains of bacteria and fungi Shown in Figure 4. The inhibiting minimal concentrations of the bacteria by the extracts indicate that the extracts generally act with low dose. Antibacterial activity was increased while increasing the concentration of extracts. The minimum inhibitory concentration is 50 µL shown high zone of inhibition.

Conclusion

Medicinal plants and their extracts were used as first medicines since ancient times. The first step of our study is the in vitro screening of three different extracts of *A. paniculata* for their biological activity. Three extracts are extracted in soxhlet using the solvent aqueous, ethanol, and acetone. The three different types of leaf extract have tested against pathogenic bacteria. Among these three extracts, ethanol derived extracts shows

high antibacterial activity against dermatophytes and MRSA bacteria due to the presence of large quantity of alkaloids, steroids, saponins and glycosides.

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