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Antibacterial potency of hydro-alcohol leaf extract of *Morinda citrifolia* L. (Noni) by soxhlet extraction method

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ABSTRACT

Morinda citrifolia L. is one of the most important traditional polynesian medicinal plant. This small evergreen tree is native of South Eastern Asia to Australia and now it has pantropical distribution all over the world. This plant has antifungal, antibacterial, anti-inflammatory and antiviral activities. During the present study, a preliminary study pertaining to hydro-alcoholic extraction of leaf extract of *Morinda citrifolia* was carried out since this method is more suitable in comparison to methanol or water extracts for clinical study. The hydro-alcoholic leaf extract was prepared by soxhlet extraction method and it was assessed with two antibiotics in-vitro using disc diffusion method against six different bacterial pathogens. In the antibacterial analysis, *Staphylococcus aureus* was found more susceptible followed by *Pseudomonas aeruginosa* and *Bacillus subtilis* towards the hydro-alcohol extract. The Amoxyclav drug was found to be impotent in order to control the growth of all bacteria but Rifamycin had good control over the pathogens. Hydro-alcoholic extract was observed the suitable drug than the amoxyclav in the present study. The results revealed that plant extracts have inhibitory activity against the tested organisms.

Keywords: *Morinda citrifolia*, Antibacterial activity, Hydro-alcoholic leaf extract, DMSO.

INTRODUCTION

India is known for its rich diversity of medicinal plants from ancient times, plants are the gift of nature to cure infectious diseases including chronic diseases [1]. *Morinda citrifolia* has pharmacological activities and traditionally used as therapeutic agents for various diseases [2]. Approximately 200 phytochemicals have been identified and isolated in different parts so far [3] and more than 160 nutraceutical compounds have been identified from this plant [4]. Its root, leaves, stem, bark, flowers and fruits are recorded as herbal medicines for different diseases. It has reported to possess hepatoprotective, anticancer, immune-modulator, anti-inflammatory, wound healing, antioxidant, anti-tubercular and wide spectrum of biological activity and is safe medicinal plant [5]. The leaves are 8-10 inches long oval shaped, dark green and shiny, with deep veins is largely used in traditional medicine and has been heavily promoted for a wide range of uses; including cough, nausea, colic, gout, tuberculosis, ring worm, arthritis, atherosclerosis, boils, burns, cancer, chronic fatigue syndrome, circulatory weakness, cold sores, congestion, constipation, diabetes, gastric ulcers, gingivitis, heart disease, hypertension and infections and the leaf of this plant is directly used on skin for ulcerations and for minor infections [6]. Moreover, the increase in antibiotic resistant bacteria is largely due to the wide and misuse of antibiotics and *Staphylococcus aureus* and few other bacteria are of the major causes of hospital-acquired infections and they never respond to any antibiotics [7]. These organisms occur naturally in the human body and lead to infections and are becoming resistant to antibiotics. In

view of new alternative therapies for handling these infectious bacteria, the present study was carried out to extract the hydro-alcoholic leaf extract by soxhlet method from *M. citrifolia* and to analyze its antibacterial activity against few bacterial pathogens.

MATERIALS AND METHODS

Collection Plant material

Mature fresh leaves of *Morinda citrifolia* plant were collected from Villianur, Pondicherry.

Preparation of plant extract

50g of fresh leaves of *Morinda citrifolia* were collected from healthy plant parts and washed two to three times with distilled water and leaves were dried in shade for 3 weeks and grounded with mechanical grinder [8], the powdered leaf material were initially defatted with 250 ml Petroleum ether and subjected to occasional shaking for 24 hrs and allowed the solvent to get evaporated completely. After complete drying, the above said residues were extracted with soxhlet extractor using hydro-ethanol as solvent [9]. The extract was filtered using whatman filter paper and dried at room temperature. The hydro-alcoholic extract yielded a dark greenish crude extract and it was preserved at room temperature and further investigation was carried out for potential of antibacterial activities.

Tested Bacterial strains

Gram negative and gram positive bacteria viz., *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Serratia marcescens* were chosen based on their clinical and pharmacological importance and used in the present study.

Antimicrobial activity

Determination of zone of inhibition method

In vitro antibacterial activity was examined by disc diffusion method [10] using hydro-alcohol leaf extract of *Morinda citrifolia*. The leaf extracts were dissolved in dimethyl sulfoxide (DMSO) and three dilutions sets (5µl, 10µl and 20µl) were made with control experiments under similar condition by using standard drugs Amoxycylav and Ampicillin for antibacterial activity, against six pathogenic bacteria *E. coli*, *S. aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Serratia marcescens*. Muller Hinton agar was used for the preparation of media plates and the bacterial culture was inoculated by sterile cotton swabs. The cultures were allowed to dry for 30 minute and the sterile filter paper discs (5 mm) with known concentration of plant extracts and control were impregnated over the surface of Mueller Hinton agar plate equidistantly. Standard antibiotic were also taken as control for comparison of the results. The zones of inhibition around the disks were measured after 24 hours of incubation at 37°C. The experiments were repeated three times and the mean values were presented (Table 1).

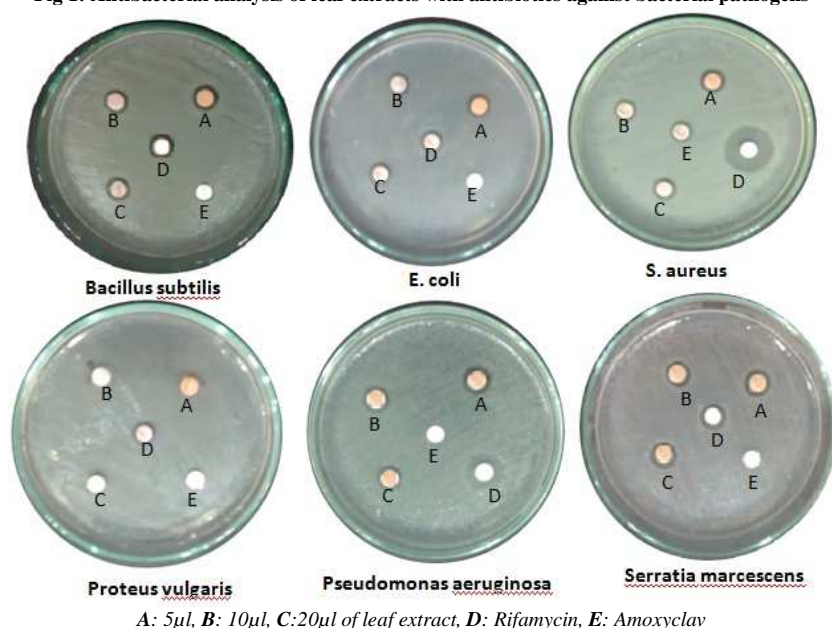
RESULTS AND DISCUSSION

The results obtained from the disk diffusion assay (Table 1) showed that the hydro-alcoholic leaf extract of *Morinda citrifolia* posses antibacterial activity against the tested organism. 10µl and 20µl dilutions of the hydro-alcoholic leaf extract had good efficacy over the pathogens (Fig 1&2). *Staphylococcus aureus* was found more susceptible followed by *Pseudomonas aeruginosa* and *Bacillus subtilis* towards the hydro-alcohol extract. It was recorded that Amoxycylav antibiotics had no control over the pathogens (Table 1, Fig 1), but Rifamycin showed good impact over the six tested organisms both gram negative and positive. The observed efficacy of the leaf extract may be due to the presence of potential phytoconstituents like phenolic compounds in the leaf extract [11]. The ethanol extract of leaf and flower of *Spathodea campanulata* was investigated by Kowti and his coworkers [12] for antimicrobial activity at 10 mg/ml concentrations by using Kirby-Bauer disc diffusion method against gram positive and gram negative organisms like *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas sps*, *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio cholera* [12]. They also compared gentamycin and streptomycin drugs with the plant extracts to find a conclusion like our present study. It was observed from the dose dependent study that the ethanol flower extract was more potent than leaf extract [12]. Getahun et al [13] in their study opined that the traditional knowledge investigated the antimicrobial activity of *Plumbago zeylanica* L. hoping to add value for the local medicinal knowledge of the plant and better efficacy than those reported before. Significant zone of inhibition was recorded from both the crude as well as fractions [13]. This study will serve as a platform for isolation of potential new novel therapeutic agents in future.

Table1: Antibacterial efficacy of *Morinda citrifolia* leaf extract against pathogenic bacteria

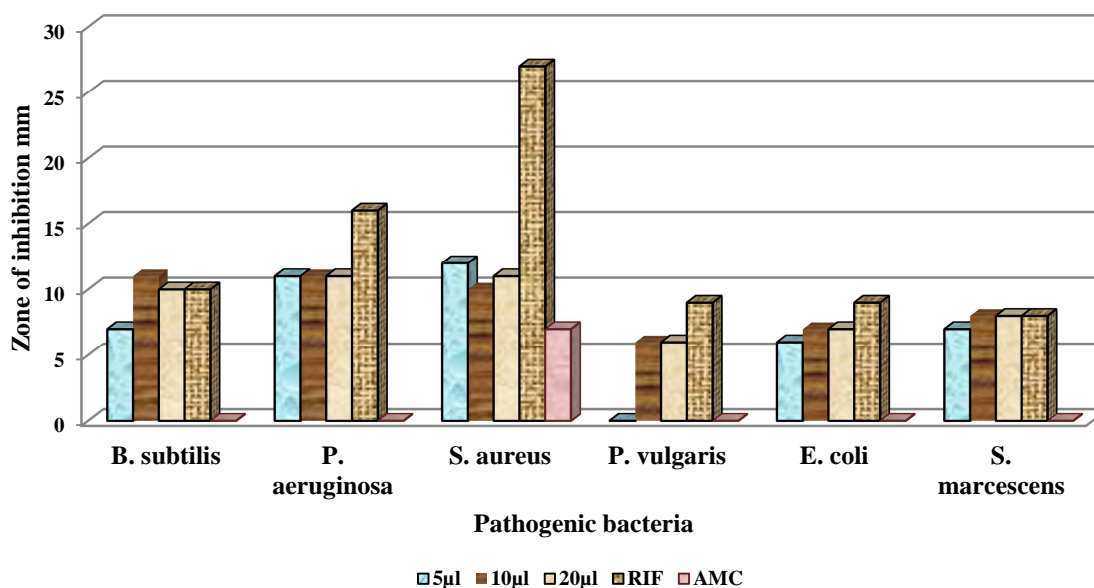
Bacterial strains	Dilutions of Soxhlet leaf extracts of <i>Morinda citrifolia</i>			Antibiotics Rif: Amoxyclav Amc: Amoxyclav	
	5 μ l	10 μ l	20 μ l	Rif	Amc
<i>Bacillus subtilis</i>	7	11	10	10	---
<i>Pseudomonas aeruginosa</i>	11	11	11	16	---
<i>Staphylococcus aureus</i>	12	10	11	27	7
<i>Proteus vulgaris</i>	---	6	6	9	---
<i>Escherichia coli</i>	6	7	7	9	---
<i>Serratia marcescens</i>	7	8	8	8	---

Fig 1: Antibacterial analysis of leaf extracts with antibiotics against bacterial pathogens



A: 5 μ l, B: 10 μ l, C: 20 μ l of leaf extract, D: Rifamycin, E: Amoxyclav

Fig 2: Susceptibility/resistance of bacterial strains towards leaf extract and antibiotics studied together



CONCLUSION

Antimicrobial activity of *Morinda citrifolia* leaf extract was compared with the standard antibiotics of against the bacterial strains. The zone of inhibition made by the hydro-alcoholic extract was found equal or greater than the zone of inhibition made by the antibiotics against the test pathogens, by which it was confirmed that the leaf extract inhibit the growth of the bacteria at low concentrations. Though there are number of antibacterial, anti-fungal drugs available in the market, they produce many side effects; hence to improve the status of therapy, plant extract of *Morinda citrifolia* may be useful for various ailments in near future. *Morinda citrifolia* leaf extract thus provides safe, easy, effective and practical solutions to every day ailments leaving behind no toxins and creating a clean, pleasant atmosphere. The overall results indicated promising baseline information for the potential uses of hydro-alcoholic leaf extract of *M. citrifolia* in the treatment of infectious disease.

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