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Research Article

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Comparative antibacterial analysis of hydro-alcoholic leaf extract of three medicinal plants by soxhlet extraction process

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ABSTRACT

Hydro-alcoholic leaf extract of three medicinal plants viz., Andrographis paniculata, Cassia alata and Morinda citrifolia were prepared by soxhlet extraction method. The antibacterial activity of hydro-alcoholic leaf extracts were carried out in-vitro using disc diffusion method against six different bacterial strains viz., gram positive (Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis) and gram negative (Escherichia coli, Serratia marcescens and Proteus vulgaris). The antibiotic potency of leaf extract was compared with the standard antibiotics, Rifamycin and Amoxyclav. Leaf extract of Andrographis paniculata was recorded with remarkable inhibition ability against the tested organisms and it was followed by Cassia alata and Morinda citrifolia. Rifamycin was found to be good drug in controlling the pathogens in comparison to Amoxyclav in our study. Pseudomonas aeruginosa didn't respond to any extracts or the antibiotics and become resistant to all at 15 µl dilution of the extract. Amoxyclav was recorded with low responsive to prevent the growth of the bacteria studied herewith.

Keywords: *Andrographis paniculata*, *Cassia alata*, *Morinda citrifolia*, Antibiotic potency, Hydro-alcoholic leaf extract, DMSO

INTRODUCTION

Utility of medicinal plants as traditional medicine is one of the common practices in India due to their wide pharmacological activities. Traditional medicines are being used at the primary health care level by many developed and developing countries [1]. In the developed countries, 25 percent of the medical drugs are based on only plants, their active substances include alkaloids, flavonoids, L-asperuloside, caproic acid, caprylic acid, phenolic compounds/essential oils and, tannins etc., [2]. Infectious diseases are the number one among all causes of death, accounting approximately one-half all deaths throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases [2]. The increase in antibiotic resistant bacteria is largely due to the widespread use and miss utilize of antibiotics in therapeutic medicine and developing the the resistance efficacy of pathogens to these antimicrobial drugs.

Andrographis paniculata is an herbaceous plant belongs to family Acanthaceae, native to India and Sri Lanka. Mostly the leaves and roots were used for medicinal purposes. *Andrographis paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India, China, Thailand, and other Asian countries [2]. It has anti-inflammatory, antiviral, antibacterial, antifungal, antithrombotic, antiatherosclerotic, antihelminthic, antiperistaltic activities. Different parts of this plant extracts are used for the prevention and treatment of common cold, hypotensive, stomachic, etc., Medicinal plants are the richest bio-resource of drugs of traditional systems of

medicine, modern medicines, neutraceutical, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [3].

Cassia alata was native to Ghana and Brazil, but it is now widely distributed in the Americas and all over Africa, Nigeria inclusive. Different parts and constituents of the plant was reported to exhibit several therapeutic properties, such as antibacterial, antifungal, antimicrobial and analgesic. The leaves of this plant are used in the treatment of ringworm. The plant is traditionally acclaimed to be effective in treating skin infections in man and animals [4]. *C. alata* leaf is also credited for the treatment of haemorrhoids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes [5]. The flowers are prepared in an infusion to treat urinary infections and used to increase urination; the leaves and stems are prepared in a decoction for ascaries and herpes ulcers.

Morinda citrifolia (Rubiaceae), *Vitex trifolia* (Verbenaceae) (Forest and Kim Starr) and *Chromolaena odorata* (L.f.) King and Robinson (synonym: *Eupatorium odoratum L.*) (Asteraceae) has a long history of use in traditional medicine for the treatment of infectious diseases including chronic diseases. *Morinda citrifolia* has pharmacological activities and traditionally used as therapeutic agents for various diseases [6]. Approximately 200 phytochemicals have been identified and isolated in different parts so far [7] and more than 160 neutraceutical compounds have been identified from this plant [8]. Its root, leaves, stem, bark, flowers and fruits are recorded as herbal medicines for different diseases it has reported to possess hepatoprotective, anticancer, immuno-modulator, anti-inflammatory, wound healing, antioxidant, anti-tubercular and wide spectrum of biological activity and is safe medicinal plant [9]. The aim of the study was to assess the antibacterial activity of hydro-alcoholic leaf extract of three plants, *A. paniculata*, *C. alata* and *M. citrifolia* prepared by Soxhlet extractor against disease causing bacteria. This study is to foster the multi dimensional use of the plant materials by integrating its antimicrobial efficacy against pathogenic microorganisms.

EXPERIMENTAL SECTION

Collection of Plant material

The healthy and fresh leaf of the plant *Andrographis paniculata, Cassia alata* and *Morinda citrifolia* were collected from Villianur area of Puducherry.

Preparation of plant extract

50g of fresh leaves of each plant, *Andrographis paniculata*, *Cassia alata* and *Morinda citrifolia* were collected and washed two to three times with tap water and distilled water and then surface sterilized with 70% of ethanol, after that leaves were dried in shade for 3 weeks and grounded with mechanical grinder [10], the powdered plant 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-alcohol of 40-60% ratio as solvent. The extracts were filtered using whatman filter paper and dried at room temperature. The hydro-alcoholic extracts yielded dark greenish crude extracts and were preserved at low temperature and further investigation for potential of antibacterial activities were carried out later on.

Tested Bacterial strains

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

Antimicrobial activity

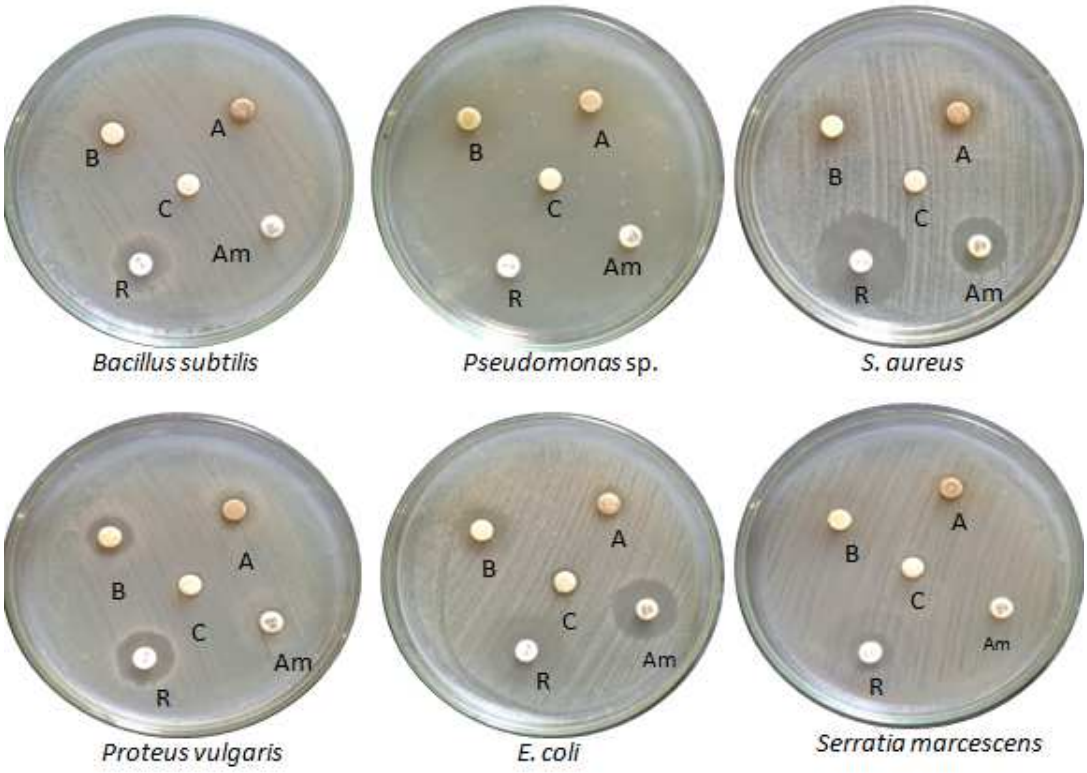
Determination of zone of inhibition method

In vitro, antibacterial activity was examined by disc diffusion method [11] using hydro-alcohol leaf extracts of *Andrographis paniculata, Cassia alata* and *Morinda citrifolia.* 20 µl of the leaf extracts were dissolved in 80 µl of dimethyl sulfoxide (DMSO) and set to 100 µl. 15 µl of the solution (leaf extract and DMSO) were taken separately were impregnated over sterile blank discs. The prepared discs were subjected to antibacterial sensitivity test against six pathogenic bacteria under similar condition by using standard drugs, Amoxyclav and Rifamycin for comparison. Muller Hinton agar (MHA) was prepared by dissolving 38g in 1000ml of distilled water and brought to boil to completely dissolve. Sterilization was achieved by autoclaving at 121^{o}C for 15 minutes [12]. Bacterial cultures were developed on MHA by using sterile cotton swabs. The cultures were allowed to dry for 5 min and the sterile filter paper discs (5mm) with 15 µl of plant extracts and control were impregnated over the surface of media plates. The

zones of growth inhibition around the disks were measured after 18 to 24 hours [7] of incubation at 37°C. For the determination of zone of inhibition against hydro-alcoholic leaf extract were screened for their antibacterial activities against the gram-positive (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) and gram negative (*Escherichia coli*, *Serratia marcescens* and *Proteus vulgaris*). The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks are calculated and compared with the controls.

RESULTS AND DISCUSSION

Fig 1: Antibacterial potency of leaf extracts and antibiotics against bacterial pathogens.
A: *A. paniculata*, B: *C. alata*, C: *M. citrifolia* leaf extracts, D: Rifamycin, E: Amoxyclav



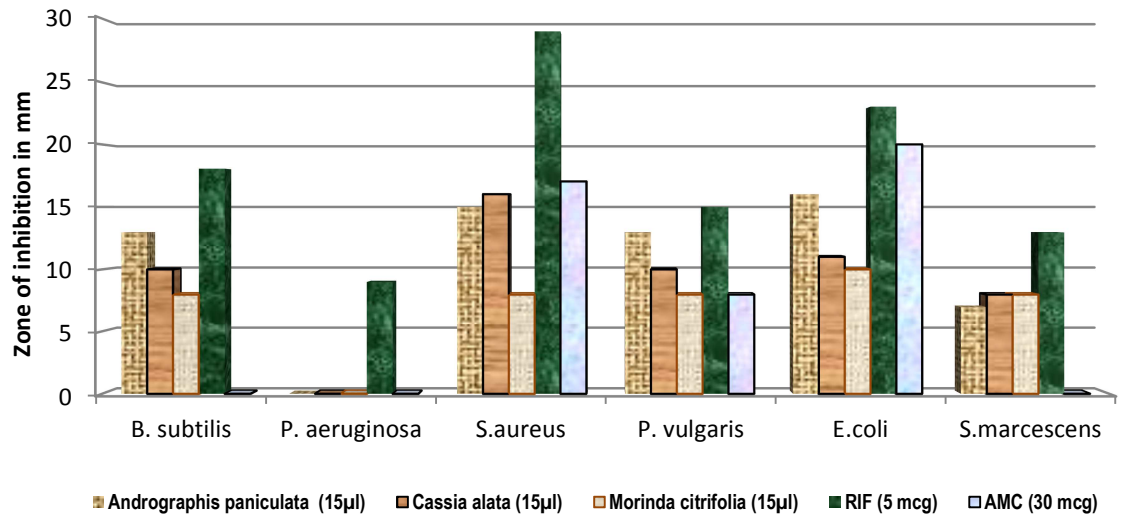
During the study period, leaf extract of *Andrographis paniculata* was recorded with remarkable inhibition ability against the tested organisms and it was followed by *Cassia alata* and *Morinda citrifolia* (Table 1, Fig 1). Between the drugs, Rifamycin was found to be good one in controlling the pathogens in comparison to Amoxyclav in our study. *Pseudomonas aeruginosa* didn't respond to any extracts or the antibiotics and become resistant to all at 15 µl dilution of the extracts (Fig 2). Amoxyclav was found with low responsive drug to prevent the growth of the bacteria studied herewith against all the bacteria. All the three plant extracts showed good zone of inhibition against the tested organisms, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* comparing to the standard antibiotic Amoxyclav. Among the studied medicinal plants, *Andrographis paniculata*, extract showed maximum antibacterial activity than *Cassia alata* and *Morinda citrifolia*. This study will serve as a platform for isolation of potential new novel therapeutic agents in future from plant materials. Equivalent to our present work, Redfern et al [13] extracted antimicrobial compound successfully by soxhlet method, they tested the compound against *E. coli* and *S. aureus* and found the result, 9 mm diameter when tested against *E. coli* and 16 mm diameter when tested against *S. aureus*. The results made by them were found similar, more or less to our results. The antibacterial assay of the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts [14]. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0mm against *B. cereus* and *S. aureus* respectively, which was agreed with our hydro-alcoholic extract.

On the basis of their finding, guava leaf-extract might be a good drug in the search for a natural antimicrobial agent [14].

Table1: Antibacterial efficacy of *Andrographis paniculata, Cassia alata* and *Morinda citrifolia* leaf extracts against pathogenic bacteria

Bacterial Pathogens	Dilutions of Soxhlet leaf extracts (15µl) Zone of inhibition (mm)			Antibiotics Rif: Amoxyclav Amc: Amoxyclav	
	Andrographis paniculata	*Cassia alata*	*Morinda citrifolia*	Rif (5 mcg)	Amc (30 mcg)
Bacillus subtilis	13	10	8	18	---
Pseudomonas aeruginosa	---	---	---	9	---
Staphylococcus aureus	15	16	8	29	17
Proteus vulgaris	13	10	8	15	8
Escherichia coli	16	11	10	23	20
Serratia marcescens	7	8	8	13	---

Fig 2: Antibacterial efficacy of *Andrographis paniculata, Cassia alata* and *Morinda citrifolia* leaf extracts



CONCLUSION

Antimicrobial activity of *Andrographis paniculata, Cassia alata* and *Morinda citrifolia* leaf extracts were compared with the standard antibiotics against the bacterial strains. It was found that the hydro-alcoholic extract showed the zone of inhibition equal or greater to the zone of inhibition of antibiotics among the tested pathogens, by which it was sure that these leaf extract surely inhibit the growth of these microorganisms at low concentrations. Though there are number of antibacterial, anti-fungal and anti-helminthic drugs available in the market, they produce many side effects; hence to improve the status of therapy, various ailments of plant extracts like *Andrographis paniculata, Cassia alata* and *Morinda citrifolia* will be much useful. All the leaf extracts 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 *Andrographis paniculata, Cassia alata* and *Morinda citrifolia* in the treatment of infectious disease.

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