



Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms

P. Arulmozhi, S. Vijayakumar*, T. Kumar

Computational Phytochemistry Lab, PG and Research Department of Botany and Microbiology, AVVM Sri Pushpam College (Autonomous) Poondi, Thanjavur (Dist), Tamil Nadu, India

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ABSTRACT

The aim of the present study is to investigate the antimicrobial potency of leaves from various extracts of *Capparis zeylanica*, *Streblus asper* and *Tribulus terrestris* were evaluated. In addition, this is the first report on MIC, MBC/MFC antimicrobial activities of above mentioned plants and also identify the phytochemical, functional groups by GC-MS and FT-IR respectively. Soxhlet extraction method was used for preparation of different extracts viz., aqueous, petroleum ether, ethyl acetate and methanol. The extracts were examined against *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Candida albicans* and *Mycobacterium tuberculosis* by agar well diffusion method, and Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) values were determined through micro dilution method. Phytochemical analysis of compounds was carried out by GC-MS analysis and functional groups were identified by FT-IR. Based on the outcome of our results, Ethyl acetate extract Showed significant antimicrobial activity against the tested pathogens especially, for *C. albicans* (40 mm) followed by ethyl acetate of *S. asper* against *S. paratyphi* (38 mm). While, the least inhibition was observed with aqueous extract of *T. terrestris* against *S. paratyphi* (10 mm). The MIC ranged from 3.21 mg/ml to 50 mg/ml and MBC/MFC 6.25 mg/ml to 50 mg/ml was recorded. Ethyl acetate extracts of almost all samples showed better activity than other extracts in inhibition growth of pathogens. Phytochemical analysis exhibited the presence of Steroids, tannins and cardiac glycosides were found only in ethyl acetate extract of *C. zeylanica*. Functional group of leaf extract was confirmed by FT-IR spectrum and GC-MS analysis of the ethyl acetate extract revealed the presence of 20 compounds. The results revealed that ethyl acetate extract of *C. zeylanica* leaves has potential activity than the other extracts as well as standard drugs (Gentamycin and Ketocozole). Hence, this plant may be recommended for further studies in isolation of active compounds and related pharmacological activities.

1. Introduction

Since ancient times, plant-derived medicines were used for the treatment of various diseases. Medicinal treatment by using plant extracts gained popularity in the late 1990s. Still plants are vital sources of medicines especially in developing countries for discovering new drugs [1]. Many efforts have been put to discover new antimicrobial components from various kinds of natural sources. Several Indian medicinal plants are having fair number of antimicrobial activity [2]. From them, new antibacterial drugs have been approved [3]. In recent years due to failure of chemotherapy an increasing emergence of antibiotic resistant organisms is in record. Therefore, it is essential to prevent the spread of these organisms and to improve the treatment methods [4]. Thus, searching not only for improved versions of existing

drugs but also for new drug targets has become an urgent need. Although the recent research in drug discovery using molecular modeling, computational chemistry and other synthetic chemical methods, natural products derived compounds are still proving to be an invaluable source of medicines for humans [5]. Recently, several studies have reported that ethnomedicinal plants are having diverse pharmacological and biological properties [6].

In this respect, three plant species (*Capparis zeylanica*, *Tribulus terrestris* and *Streblus asper*) were selected based on the ethnomedicinal information of previous literature. *C. zeylanica* Linn (*C. zeylanica* Lin, *C. brevispina* DC.) belonging to Capparidaceae family which is familiarly known as Indian caper. The plant is a stiff shrub having large branches and spreaded over in Bangladesh, Sri Lanka, Malaysia [7,8]. In the traditional ayurvedic system of medicine, this shrub is used as

* Corresponding author.

E-mail address: svijaya_kumar2579@rediff.com (S. Vijayakumar).

“Rasayana drug”. Leaves are used as counter-irritant, febrifuge and as cataplasm to treat swellings and piles in North India [9]. Most of the parts like root, bark, fruit, leaves and seeds are used for the treatment of various ailment categories.

T. terrestris L. is commonly grown in Africa, Southern Europe, China, Japan, Korea and western parts of Asia [10]. It grows well in light textured soils and wide range of soil types. Commonly, it can be found in cultivated crops, overgraze pastures, roadsides, lawns and neglected areas. Traditionally, various kinds of wounds were treated by the leaves of *T. terrestris*. This plant is having an enormous amount of calcium [11], and extracts have immunostimulatory and antimicrobial effect [12].

S. asper Lour, belonging to the family of Moraceae, is a medicinal plant which spread over in various Asian countries, namely India, Southern China, Malaysia, the Philippines and Thailand. This plant has been used for many pharmaceutical purposes for various diseases. Its leaf extract has been tested for toxicity, mutagenicity, antimutagenicity and antimicrobial activity [13].

In the present study, *C. zeylanica* leaf extract of different solvents were tested against selected human pathogens. This is the first report on MIC, MBC/MFC antimicrobial activity of *C. zeylanica* leaf extract. And also leaf extract of *T. terrestris* and *S. asper* in different solvents like aqueous, petroleum ether, ethyl acetate and methanol were tested against *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Candida albicans* and *Mycobacterium tuberculosis*. Till now the investigation of phytochemicals by GC-MS and functional groups of FT-IR has not been studied from *C. zeylanica* leaf extract.

2. Materials and methods

2.1. Plant material

Plant materials of three plant species viz, *C. zeylanica*, *T. terrestris*, and *S. asper* were selected, mainly based on the ethno medicinal properties (Supplementary data I). The grownup green and good leaves were gathered from the inside and outside the A.V.V.M Sri Pushpam College, Poondi campus Thanjavur, Tamil Nadu, India. The collected plants were authentically identified with the help of floras, such as Flora of Presidency of Bombay [14], Flora of British India, Flora of presidency of Madras [15] and Flora of Karnataka [16].

2.2. Preparation of extracts

The dried parts of the leaves were pulverized through a mechanical grinder. The powdered material was dried in hot air oven at moderate temperature. The powdered material was subjected to successive Soxhlet extraction by various solvents namely aqueous, petroleum ether, ethyl acetate and methanol were used. After that, extract was concentrated and stored at 4 °C until further use in the equipment [17].

2.3. Microbial cultures

Antimicrobial potency of each plant extract was evaluated using six bacterial and one fungal strain causing human infectious diseases. The organisms selected were *Staphylococcus epidermidis* MTCC 2639, *Enterococcus faecalis* MTCC 439, *Salmonella paratyphi* MTCC 735, *Shigella dysenteriae* (Lab isolate from stool), *Mycobacterium tuberculosis* (H 379) and *Candida albicans* (MTCC 227) were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India through Eumic Analytical Lab and Research Institute, Trichy, India.

2.4. Inoculum preparation

Bacterial strains were sub cultured on overnight at 37 °C in Muller-Hinton agar slants. The colonies were transferred using 5 ml of sterile

saline water, standardized at 350 nm (equivalent to harf McFarland) which gave a stock suspension of microorganisms equal to 1×10^6 CFU/ml saline. Sabouraud dextrose agar (SDA) was used for sub-culturing *C. albicans* at 27 °C for 24hr. As previously mentioned bacterial pathogens a suspension of 1×10^5 CFU/ml saline was prepared.

2.5. Antimicrobial assay

Agar well diffusion was used to evaluate the antimicrobial activity of each plant extract as reported by Valgas et al., [18]. Similarly to the procedure used in disk diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, the wells were dispensed with the respective leaf extracts (20–100 ml) at preferred concentration. *Gentamycin* (5 µg) and *Ketoconazole* (5 µg) as positive controls, solvents as negative control were used. Then, agar plates were incubated for 24 h at 37 ± 2 °C for bacterial strains and 30 ± 2 °C for 72–96 h for fungal strain. The assay was carried out in triplicates. The measurement of zone inhibition was taken from the top of the well to the clear zone in millimeter (mm).

2.6. Minimum inhibitory, minimal bacterial and fungicidal concentrations

Minimum inhibitory concentration (MIC) of various extracts of leaves of selected plant leaves was determined by the micro dilution method using 96 microwell plates (Flat Bottom; polystyrene, Eppendor). Before start up of the experiment, 100 µl of different concentration of crude extracts of selected plants (3.12, 6.25, 12.50, 25.0 and 50.0 µg/ml) were dissolved in various solvents. After that, every experimental extract was dispensed individually in 6 wells of 96 microwell plates. Then the plates were dried under sterile state for 2 h in order to evaporate the solvents. The plates were then added with 100 µl of each bacterium and fungus separated under aseptic state. For the growth of bacteria and fungi they were incubated for 24–48 h at 37 ± 2 °C and for 48–96 h at 27 ± 2 °C respectively. MIC of bacteria and fungus was determined following the method of Suffredini et al., [19]. In general, culture starting from each MIC test, dilution was placed on fresh plates of plates of MHA and SAD for bacterial and fungal strains respectively. Both of them were incubated for 27 and 72hrs respectively at 37 ± 2 °C. There is no sign of growth of bacterial and fungal strains, particularly in MHA and SDA plates at least concentration, So that MHA and SDA plates was recognized as MBC/MFC values.

2.7. Phytochemical analysis

The phyto-chemical analysis of each plant extract was analyzed for the presence of alkaloids, steroids, saponins, flavonoids, tannins, terpenes, phenol compounds and cardiac glycosides using standard methods [20,21].

2.8. FTIR analysis

The dried leaf powder of ethyl acetate extract subjected to identifying the functional groups were carried out by Fourier Transform Infrared Spectrophotometer (FT-IR Model/model: IF 25, Bruker Germany) [22]. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powdered sample of extract was loaded in FT-IR spectroscope, with a scan range from 400 to 4000 cm^{-1} with resolution of 4 cm^{-1} [23].

2.9. GC-MS analysis

GC-MS analysis of ethyl acetate extract showing most promising antimicrobial activity was carried out using Perkin Elmer Clarus 680 gas chromatography mass spectrophotometer provided with a FID

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