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Research Article

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Screening of Antimicrobial Activity of *Cymbopogon caesius* and *Cymbopogon coloratus* Essential Oils

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Abstract

The present study is aimed at extracting the essential oil from *Cymbopogon caesius* and *Cymbopogon coloratus* and to check the susceptibility of Citrobacter, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella enterica* ser. typhi, *Shigella flexneri*, *Candida albicans*, *Trichophyton rubrum*, *Aspergillus niger* and *Aspergillus fumigatus* to the extracted essential oils. The essential oil was obtained by steam distillation of leaves of *Cymbopogon caesius* and *Cymbopogon coloratus*. The agar diffusion method using filter paper disks was employed to assess the antibacterial activity and antifungal activity.

Keywords: *Cymbopogon caesius*, *Cymbopogon coloratus*, Essential Oils, Hydro Distillation, Antimicrobial Activity

Introduction

The genus Cymbopogon belongs to the family, Poaceae (or Gramineae). The Poaceae family has about 700 genera and 11,000 species widely distributed in all regions of the world especially in south East Asia. Cymbopogon is native to warm temperate and tropical regions. The word cymbopogon was introduced by Sprengel in 1815. At that time the genus consisted of a few species. They are then moved to the genus Andropogon and it is the sub type of Graminaeae.

Natural essential oils from plant sources are potent and safe due to their harmless nature and minimal or no side effects which are beneficial than the artificial ones[1]. The essential oils are used in perfumes, flavoring, cosmetic and pharmaceutical preparations[2]. The oils from some of the species are used in treatment of various human ailments such as cough, fever, gout, leprosy, stomach disorders and have sedative properties[3,4]. The essential oils that are derived from Cymbopogon plants are of medicinal importance[5]. The organic solvent extracts of Cymbopogon plants have antimicrobial properties. The oils of some Cymbopogon species have powerful germicidal and antibacterial properties[6]. The spentmare of Cymbopogon is a rich source of lignocellulosic material, which is used for the manufacture of fiber boards and paper pulp[7].

The whole plant materials of Cymbopogon citrates are recommended to be taken because it has many beneficial effects in human health[8]. The essential oils of the grasses of species of Cymbopogon have an industrial profile. They are used in beverages, food stuffs, fragrances, house hold products, personal care products, pharmaceuticals and in tobacco[9]. Cymbopogon plant extract has been used traditionally to treat a number of infectious diseases including those caused by bacteria, fungi, protozoa and viruses. The aim of the present study was to extract the essential oils from *Cymbopogon caesius* and *Cymbopogon coloratus* and to evaluate the antibacterial and antifungal activity of the essential oils.

Materials and Methods

Collection and Identification of Samples:

The leaves of *Cymbopogon caesius* and *Cymbopogon coloratus* were collected from Acharya Nagarjuna University located NH-5 between two cities Vijayawada and Guntur, Andhra Pradesh. The identification of the plants was done by the senior botanist, department of botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh.

Extraction of Essential Oil from the Leaves:

The extraction of essential oil may sound only to be of technical interest. It is one of the points which determine the quality of the oil. The essential oils are present in the oil glands, oil salts and glandular hairs of the plants. The essential oil from aromatic plants of Cymbopogon can be obtained either hydro distillation or steam distillation. Hydro distillation is cheaper than steam distillation. Therefore hydro distillation method was commonly used for the extraction of essential oil. Clevenger apparatus is used for distillation process. The sample was submitted for 3 to 4 hours to hydro distillation using a Clevenger apparatus. The distilled oil should be left to stand for few hours. Anhydrous sodium sulphate (approximately 3%) is added to remove moisture. The oil was stored at -4ºC until tested and analyzed.

Test Organisms:

The test organisms were procured from microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, Punjab. Gram negative bacterial strains such as *Citrobacter* (MTCC6805), *Klebsiella pneumoniae* (MTCC9544), *Proteus mirabilis* (MTCC9493), *Salmonella enterica* ser. typhi (MTCC8767) and *Shigella flexneri* (MTCC1457) were used as test organisms for antibacterial activity experiments. Fungal strains such as *Candida albicans* (MTCC7533), *Trichophyton rubrum* (MTCC3018), *Aspergillus niger* (MTCC1344), *Aspergillus fumigatus* (MTCC2483) were used as test organisms for antifungal activity experiments.

Preparation of Inoculum:

Stock cultures of bacterial and fungal strains were maintained at 4°C on slopes of nutrient agar and sabouraud dextrose agar, respectively. Active cultures for experiments were prepared by transferring a lapful of cells from the stock cultures to test tubes of Mueller-Hinton broth for bacteria and Sabouraud dextrose broth for fungi that were incubated without agitation for 24 hours at 37°C and for 48 hours at 25°C, respectively. Activated cultures of bacterial and fungal strains in Mueller-Hinton broth for bacteria and Sabouraud dextrose broth, respectively were adjusted to 108CFU/ml.

Determination of Antimicrobial Activity:

The antimicrobial tests were carried out by the disc diffusion method[10].

Antibacterial Activity:

The antibacterial activity was screened by using Mueller-Hinton Agar. The Mueller-Hinton Agar plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5min. 100µl of bacterial inoculum suspension was swabbed uniformly. The inoculum was allowed to dry for 5min. Sterile filter paper discs (5mm diameter), previously impregnated and saturated with the *Cymbopogon caesius* and *Cymbopogon coloratus* essential oil were placed on the surface of medium. The compound was allowed to diffuse for 5min. The plates were incubated at 37°C for 24 hours. Tetracycline (20µg/disc) was used as positive control. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. The antibacterial studies were performed in duplicate.

Antifungal Activity:

The antifungal activity was screened by using sabouraud dextrose agar. The sabouraud dextrose agar plates were prepared by pouring 15ml of molten media into sterile petriplates. The plates were allowed to solidify for 5min. 100µl of fungal inoculum suspension was swabbed uniformly. The inoculum was allowed to dry for 5min. sterile filter paper discs (5mm diameter) were impregnated with essential oils extracted from *Cymbopogon caesius* and *Cymbopogon coloratus* and dried. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5min. The plates were incubated at 27°C for 48hours. As a positive control, Nystatin (20µg/disc) was used. Antifungal activity was evaluated by measuring zones of inhibition of fungal growth. The Antifungal studies were performed in duplicate.

Results and Discussion

The hydrodistillation of leaves of *Cymbopogon caesius* and *Cymbopogon coloratus* produced 2.3ml of essential oil. *Cymbopogon caesius* and *Cymbopogon coloratus* essential oils exhibited antibacterial and antifungal activity against all the tested bacterial [Gram negative bacterial strains-*Citrobacter* (MTCC6805), *Klebsiella pneumoniae* (MTCC9544), *Proteus mirabilis* (MTCC9493), *Salmonella enterica* ser. typhi (MTCC8767) and *Shigella flexneri* (MTCC1457) and fungal strains [*Candida albicans* (MTCC7533), *Trichophyton rubrum* (MTCC3018), *Aspergillus niger* (MTCC1344), *Aspergillus fumigatus* (MTCC2483)], respectively. The results are presented in Table.1, Fig.1 and 2.

Antimicrobial activity of palmarosa oil was tested against gram positive and gram negative microbial strains and fungal organisms. Bacillus strains were found to show high sensitivity to essential oils. Species of *Aspergillus* in fungal organisms showed high sensitivity[11].

Fig.1: Antimicrobial activity of *Cymbopogon caecius* essential oil, *Citrobacter*

Fig. 2: Antimicrobial activity of *Cymbopogon colaratus* essential oil, *Klebsiella pneumoniae*

Conclusion

The present study is the first to demonstrate the antibacterial and antifungal activities of *Cymbopogon caesius* and *Cymbopogon coloratus* essential oils against the selected gram-negative bacteria, gram-positive bacteria and fungal species. Thus, it appears that essential oils of *Cymbopogon caesius* and *Cymbopogon coloratus* act as a natural antibacterial and antifungal agent.

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Table 1: Antimicrobial activity of *Cymbopogon caesius* and *Cymbopogon coloratus* essential oil

Sample	1	2	3	4	5	6	7	8	9
Cymbopogon caesius	22mm	19mm	21mm+	19mm	23mm	21mm	21mm	23mm	25mm
Cymbopogon Colaratus	23mm	20mm	22mm	18mm	21mm	24mm	22mm	24mm	21mm

Bacterial strains: 1. *Citrobacter; 2. Klebsiella pneumonia; 3. Proteus mirabilis; 4. Shigella flexneri; 5. Salmonella enterica ser. typhi*
Fungal strains: *6. Candida albicans*; 7. *Trichophyton rubrum*; 8. *Aspergillus niger; 9. Aspergillus fumigatus*

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