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Research Article

COMBINED ANTIMICROBIAL ACTIVITY OF LEMON GRASS OIL AND TULASI OIL

K. Revathi*, C.K. Ashok Kumar, K. Thamizhvanan

*Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy, Tirupathi, Andhra Pradesh, India.

ABSTRACT

The aim of this study was to investigate the antimicrobial activity of volatile oils obtained from *Cymbopogon citrates* and *Ocimum sanctum* against *Staphylococcus aureus* (bacteria) and *Aspergillus niger* (fungi) by agar disc diffusion method and turbidimetry method. The results showed that the maximum antimicrobial activity was shown by lemon grass oil than the ocimum oil. The mixture of these two oils shows maximum antimicrobial activity than the individual oils. As these oils showed antifungal and particularly high antibacterial activity against the test strains. These results support that the mixture of these plants oil were used to treat bacterial infections.

Key Words: Essential oils, Hydro distillation, Antimicrobial activity, Inhibition zone.

INTRODUCTION

Medicinal plants are the richest bio resource of drugs for traditional systems of medicine. The majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. However, the potential of higher plants as source for new drugs is still largely explored [1].

Medicinal plants containing essential oils in higher amounts shows antibacterial and antifungal activities [2]. Natural essential oils from plant sources are potent and safe due their harmless nature and minimal or no side effects which are beneficial than the artificial ones[3].volatile oils are widely used as analgesic, antibacterial, deodorizing, febrifuge, fungicidal, antiseptic, antidepressant, astringent, diuretic, galactogogue, insecticidal, antipyretic, antimicrobial and sedative properties. It finds utility in many areas due to these properties [4].

The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease. This situation forced the researchers to search for new antimicrobial substance from various sources including medicinal plants [5]. The aim of this study was to assess the antibacterial and antifungal activity of two essential oils lemon grass oil and tulasi oil individually and in combination against *Staphylococcus aureus* (bacteria) and *Aspergillus niger* (fungi) [6]. This study was used to develop antimicrobial drugs [7].

MATERIALS AND METHODS

Plant sample collection

The leaves of lemon grass and tulsi were collected wildly from the tirumala hills, were authentified as *Cymbopogon citrates* and *Ocimum sanctum* by the taxonomist, Department of Botany in S.V University, Tirupati(Reg No:10606 and Reg No:10643) and worcher specimen is stored in our laboratory for future reference.

Extraction procedure

The essential oils are present in the oil glands, oil sacks and glandular hairs of the plant [8]. Therefore, before distillation, the day wilted plant material is cut into small pieces enable them to expose directly as many oil glands as is possible [9]. Once the plant material has been reduced in size, it must be distilled immediately to avoid oil loss.

Clevenger apparatus is used for distillation process [10]. The wilted leaves are steam distilled which takes about 3 hours. Dipping the chopped lemongrass or Ocimum *sanctum* leaves in sodium chloride solution for 24 hours at 1-2 % concentration before distillation has been found to increase the citral content and eugenol content of lemon grass and tulasi respectively [11].

Corresponding Author: **K. Revathi** Email; itsrevati.43@gmail.com

Preparation of combined mixture of lemon grass oil and tulasi oil

Combined mixture of lemon grass oil and tulasi oil was prepared by mixing equal volumes of lemon grass and tulasi oil.

Test organisms

Microorganisms were obtained from the department of microbiology, Sree Vidyanikethan College of Pharmacy, A. Rangampet, Tirupati. One strain of gram positive bacteria *Staphylococcus aureus* and one strain of fungi *Aspergillus niger* were sub cultured and used for the antimicrobial activity.

EXPERIMENTAL METHOD

Preparation of inoculum

The bacterial and fungal strains were preserved in the nutrient agar at 4^0c were revived in nutrient broth (liquid medium) and incubated at 37 ± 1^0c for over night were used for the study.

Determining the antimicrobial activity

Turbidity method: The antimicrobial activity is determined by the formation of turbidity. It is used to find out Minimum Inhibitory Concentration [12]. For tubidimetric assays specialized tubes are used called assay tubes. Concentration of 24 hours broth culture can be found out by serial dilution [13]. Suitable concentration of culture i.e *Staphylococcus aureus* (bacterial culture) and *Aspergillus niger* (fungal culture) were selected. The selected concentration of culture is called seed broth. 0.2ml of drug (i.e lemon grass oil, ocimum oil, and equal mixtures of both) each are mixed with 0.8ml of seed broth which is serially diluted. Find out, where the turbidity forms in highest dilution, that concentration is called Minimum Inhibitory Concentration (MIC), there by the drug has activity against the *Staphylococcus aureus* (bacteria) and *Aspergillus niger* (fungi).

Agar disc diffusion method: The nutrient agar is melted, cooled suitably, poured in to Petri dishes. Spread 0.2ml of known concentration of inoculum i.e *Staphylococcus aureus* (bacteria) and *aspergillus niger* (fungi) on the surface of the solidified agar in two different petri dishes (spread plate technique).The antimicrobial discs of lemon grass oil, oscimum oil and equal mixtures of both oils are placed by using sterile forceps over the agar plate at least 15mm from the edge of the plate [14]. Now the disc is gently pressed to give better contact with agar. The plates are incubated at 37^0c for 24 hours. Antimicrobial potency is found out based on the formation of zone of inhibition [15].

RESULTS AND DISCUSSION

Antimicrobial activity of lemon grass oil and tulasi oil were determined by using both turbidimetry method and disc diffusion method.

Figure 1. Shown clarity (bacteria had been killed by the given mixture of lemon grass oil and Tulasi oil).

Figure 2. Shown clarity (fungi had been killed by the given mixture of lemon grass oil and Tulasi oil).

In turbidimetry method no tubidity was observed which determines the lemon grass oil and oscimum oil has antimicrobial activity. Anti-microbial activity of lemon grass oil and ocimum oil was determined by the application of disc diffusion method against *Aspergillus niger* (fungi) and *Staphylococcus aureus* (bacteria).

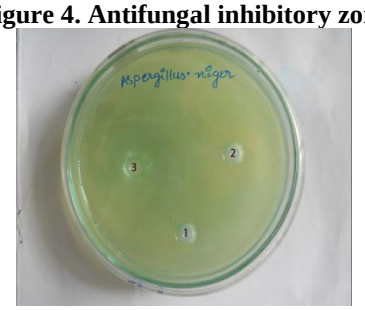
Figure 1

Figure 2

Figure 3. Antibacterial inhibitory zone

1. Inhibitory zone of tulasi oil
2. Inhibitory zone of lemon grass oil
3. Inhibitory zone of mixture of lemon grass oil and tulasi oil

Figure 4. Antifungal inhibitory zone



1. Inhibitory zone of tulasi oil
2. Inhibitory zone of lemon grass oil
3. Inhibitory zone of mixture of lemon grass oil and tulasi oil

Table 1. Antimicrobial activity as diameter of inhibition zone (mm) of the tested essential oils 50 ±µl/hole against fungi and bacteria isolated from the leaves of *Cymbopogon citrates* and *Ocimum sanctum*

Sample	Inhibition zone (mm)	
	Against bacteria (*Staphylococcus aureus*)	Against fungi (*Aspergillus niger*)
Lemon grass oil	43±3	18±0.5
Ocimum oil	37±3	8±0.5
Lemon grass oil and Ocimum oil (in equal ratio)	46±3	21±0.5

CONCLUSION

In this study the lemon grass oil and tulasi oil were extracted from the plant sources *Cymbopogon citrates* and *Ocimum sanctum* respectively showed antimicrobial activity. Lemon grass oil showed more susceptibility than the Tulasi oil to *Aspergillus niger* (fungi) and the equal mixture of both these oils showed more susceptibility than the two individual oils. Lemon grass oil showed more susceptibility than the Tulasi oil to *Staphylococcus aureus* (bacteria) and the equal mixture of both these oils showed more susceptibility than the two individual oils. Finally the equal mixture of lemon grass oil and tulasi oil showed more susceptibility to bacterial culture (*Staphylococcus aureus*) than the fungal culture (*Aspergillus niger*. Finally it is concluded that the mixture of lemon grass oil and tulasi oil are used to treat bacterial infections.

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