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Antimicrobial activity of essential oils against certain bacterial species

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Abstract
Essential oil extracted from plants not only used as drugs but also for controlling different insects and as medicine to prevent skin diseases. Plants have supplied over 25% of prescription drugs used in human medicine and such pharmacologically active plants have also lead to natural pesticide. The present study is carried out to check the antibacterial activities of different essential oils namely Black seed oil, Castor oil, Clove oil, Mustard oil and Neem oil on different bacterial species viz. *Bacillus cereus, Lactobacillus acidophilus, Pseudomonas fluorescens, Proteus vulgaris* and *Staphylococcus lactis* through disc diffusion method and their zone of inhibition was measured using zone measuring scale. Among all the bacterial strains *Lactobacillus acidophilus* resulted the lowest antibacterial activity against all the essential oils. All the essential oils showed maximum to minimum antimicrobial activity against all the bacterial species and some also no activity.

Keywords: Antimicrobial, Antibacterial, Disc Diffusion, Drugs, Essential oils, etc.

1. Introduction
Plants are the oldest friend to mankind. People in the prehistoric times used plant quite interactively for food, shelter, and even for curing their many bodily disorders and thereby kept health in perfect state of fitness and lived a long life. The medicinal plants play a very important role for time immemorial among the literates and the highly civilized people in the folk lores, superstitious traditions, various rituals, wearing of amulets, witch crafts, and chanting of mantras connected with healing of disease.
Ancient literatures also mention herbal medicines for age related disease namely memory loss, osteoporosis, diabetic wounds, immune and liver disorder etc. for which no medicine or only palliative therapy is available. These drugs are made from renewable resource of raw material by eco-friendly process and will bring economic prosperity to the masses growing these raw materials
The beginning of therapy by chemicals has lost in antiquity. Due to the indiscriminate use of commercial antibiotics, multiple drug resistance of has developed now a days. During the last ten years, pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance has increased astronomically [1].
All oils tested to date have displayed some antimicrobial activity and some have been shown to be more effective than others. Thymol, carvacrol, linalool and eugenol are main constituents of some plant essential oils that have been shown to have a wide spectrum of activity against microbes [2, 3].
Plants and their essential oils are potentially useful source of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food borne pathogens [4-7]. The main constituent of essential oils mono and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes, and ketones are responsible for the biological activity [7].
Food processors, food safety researchers, and regulatory agencies have been increasingly concerned with the growing number of food-borne illness outbreaks caused by pathogens like *Staphylococcus aureus*, *Salmonella* sp., *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and entero-pathogenic *Escherichia coli*. These bacteria cause over 90% of all cases of food poisoning.
The present research study was carried out with the following objectives:
1. To study the antimicrobial activities of the essential oils against the different species of the bacteria namely *Bacillus cereus, Lactobacillus acidophilus, Pseudomonas*

fluorescens, *Proteus vulgaris, Staphylococcus lactis.*

2. To do disc diffusion method for the essential oils with the bacterial species and check their zone of inhibitions.

2. Materials and Method

The essential oils taken for this work were Castor oil, Clove oil, Black seed oil, Neem oil, and Mustard oil.

2.1. Test Organisms

Microorganisms for the study were obtained from the laboratory of Department of Microbiology, Annamalai University.

The following organisms (bacteria) were used: *Bacillus cereus, Lactobacillus acidophilus, Pseudomonas fluorescens, Proteus vulgaris, and Staphylococcus lactis.* The stock cultures of Microbial strains were maintained in nutrient agar slants at 4 ºC at refrigerator.

2.2 Preparation of media for antimicrobial activity

The following media were prepared and used for testing of antimicrobial activities

1. Nutrient agar
2. Mueller Hinton agar

pH was adjusted to neutral. The media was sterilized at 121 ºC for 15 min at 15 psi and poured in sterile petridishes in laminar air flow to avoid contamination.

2.3. Evaluation of antimicrobial activity of essential oil

2.3.1. Preparation of inoculum

The organisms to be used for the in vitro test were maintained and preserved on nutrient agar slants by refrigerator at 4 ˚C. The organisms were impregnated over the nutrient agar plates directly with the help of an inoculum loop from the pure culture slants. The cultures were spreaded over the plates with the help of a L rod and then kept for drying.

2.3.2. Preparation of filter paper disc

Discs of 5mm diameter were prepared using Whatman filter paper No.1. These were sterilized at hot air oven at 160 ºC for 1hour. The discs were impregnated with the different essential oils taken for the study. Then the discs were stored at 4 ºC for future use.

2.3.3. Disc diffusion method

Antimicrobial activity of essential oil is tested through Disc diffusion method. Disc diffusion method is most commonly employed method to evaluate the antimicrobial activity. The disc diffusion technique widely used in Kirby-Bauer Method. Petriplates were prepared by pouring 20ml of Nutrient agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standard inoculum suspension was poured and uniformly spread. The excess inoculum was drained of and the plates were allowed to dry for 5 minute. After drying the disc extracts were placed on the surface of the plates gently with the help of a sterile forceps and pressed gently ensured that no contact with the inoculated agar surface. The inoculated plates were incubated at 37^0C for 24 hours, the zone of inhibition was observed and measured in mm.

3. Results

3.1. Antimicrobial activity of the essential oils against the bacterial species

Table 1

Sample	Test microorganisms with Zone of inhibition(mm)				
	Staphylococcus lactis	***Bacillus cereus***	***Lactobacillus acidophilus***	***Pseudomonas fluorescence***	***Proteus vulgaris***
Mustard oil	10	9	Nil	8	9
Clove oil	22	19	20	24	17
Castor oil	7	8	Nil	8	7
Black seed oil	13	12	14	9	17
Neem oil	20	9	8	Nil	Nil

4. Discussion

In the past few years there has been a resurgence of interest in study of the medicinal and antimicrobial activity of the different essential oils to check their potential regarding the microorganisms and therapeutic value. The essential oils are gaining a huge importance as an antimicrobial compounds during recent few years.

Traditional medicinal methods, especially the use of medicinal plants still play a vital role to cover the basic health needs in the developing countries [8]. Testing microorganisms for their susceptibility to antimicrobial activity is a common laboratory procedure that serves as an important aid to chemotherapeutic investigation in the case of infections

The present study was conducted to find out the antimicrobial activity of the different essential oils against the different bacterial strains. The different essential oils taken for the study were Castor oil, Clove oil, Black seed oil, Neem oil, and Mustard oil. Among all the essential oils taken Black seed oil and Clove oil resulted the highest antibacterial activity. The Mustard essential oil resulted the least antibacterial activity.

The following bacterial species were used: *Pseudomonas fluorescens, Staphylococcus lactis, Proteus vulgaris, Lactobacillus acidophilus, and Bacillus cereus.*

Among all the bacterial strains *Lactobacillus acidophilus* resulted the lowest antibacterial activity against all the essential oils. All the essential oils showed more or little antimicrobial activity against all the bacterial species and in some cases it gave no result.

5. Activity of the different essential oils against the test microorganisms.

5.1. Activity of the Black seed essential oil against the test microorganisms

The black seed essential oil showed the highest zone of inhibition in *Proteus vulgaris* with 17 mm. *Bacillus cereus, Lactobacillus acidophilus,* and *Staphylococcus lactis* also resulted zone of inhibitions. *Pseudomonas fluorescens* shows the lowest zone of inhibition.

The antimicrobial activity of black seed oil is may be due to the presence of several compounds. It may be due to the presence of Linoleic and Linolenic acid i.e. Omega-3 and Omega-6. A similar report was done by [9]. It may be also due to the presence of melanthin, thymoquinone, nigilline and tannins in the oil. Similar report was forwarded by [10].

5.2. Activity of the Castor essential oil against the test microorganisms

Pseudomonas fluorescens and *Bacillus cereus* resulted a similar zone of inhibition of 8 mm respectively. *Staphylococcus lactis* and *Proteus vulgaris* resulted a very low zone of inhibitions of 7 and 6 mm respectively. *Lactobacillus acidophilus* resulted no zone of inhibition.

Camphor as well as 1,8-cineole was revealed to inhibit the growth of bacteria and fungi. Therefore, the detected antimicrobial properties of this essential oil could be due to the relatively high concentration of α-pinene (16.88%), which is believed to actively inhibit the growth of microorganisms [3].

The antimicrobial activity of the castor oil may be due to presence of ricinoleic acid. A report was given by [11]. It may be also due to the presence of ricin or other castor oil derivatives such as Kolliphor EL.

5.3. Activity of the Clove essential oil against the test microorganisms

Pseudomonas fluorescens resulted the highest zone of inhibition of 24 mm. *Staphylococcus lactis, Bacillus cereus*, and *Lactobacillus acidophilus* also resulted a zone of inhibition of 22, 19 and 20 mm respectively. *Proteus vulgaris* resulted the lowest zone of inhibition of 17 mm respectively.

The antimicrobial activity of clove oil is may be due to the presence of eugenol, eugenie lactate, and caryophyllene respectively earlier reported [12].

5.4. Activity of the Mustard essential oil against the test microorganisms

Staphylococcus lactis showed the highest zone of inhibition of 10 mm respectively. *Lactobacillus acidophilus* showed no zone of inhibition. Other species resulted little zone of inhibition in mustard oil.

The antimicrobial activity of mustard oil may be due to presence of euric acid and oleic acid. A similar report was given by [13]. Also may be due to presence of Omega-3 and Omega -6 acids.

5.5. Activity of the Neem essential oil against the test microorganisms

The neem essential oil showed the highest zone of inhibition in *Staphylococcus lactis* with 10 mm. *Bacillus cereus* and *Lactobacillus acidophilus* also showed zone of inhibition of 9 and 8 mm.

The antimicrobial activity of neem oil may be due to the presence of azadirachtin. It may be also due to the steroids compounds present in the oil. A similar report was given by [14]. The neem oil resulted no zone of inhibition with *Pseudomonas fluorescens* and *Proteus vulgaris.*

Several constituents of *N. sativa* like essential oil, thymoquinone and hydrothymoquinone which are components of the essential oil, alkaloids and saponins were qualitatively reported to possess antibacterial activity [15].

6. Conclusion

The ethno medicinal study of plants is important for modern day medicine but its usefulness cannot be overemphasized if methods are not standardized to obtain comparable and reproducible results. Although extensive research have been done within this field, an essential oil may contain 40 or more different identifiable component chemicals which makes it difficult to determine the active components. The results of the present study further demonstrated that Black seed oil, Castor oil, Clove oil, Mustard oil and Neem oil possess varying degree of antimicrobial activity. These essential oils act through their natural inhibitory mechanisms by either inhibiting or killing the pathogens completely. However, extensive researches are necessary to search for active principles responsible for these activities.

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