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ANTIMICROBIAL ACTIVITY OF OILS EXTRACTED FROM DIFFERENT MEDICINAL PLANTS.

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ABSTRACT

Objective: It has been postulated that geographical locations of the herbs affect the constituents of their essential oils and thus the degree of their antimicrobial action. This study examine Six different oils extracted i.e. fresh carpel oil of *Zanthoxyulum rhetsa* Roxb DC, Dried carpel oil of *Zanthoxyulum rhetsa* Roxb DC, Eucalyptus oil, Clove oil, Palmarose oil and Patchouli oil to determine the antimicrobial potential of their extracted oils. **Method:** The active agents in each plant were extracted by steam distillation and by boiling. The antimicrobial activities of the extracts were determined at neat and by two-fold dilutions in well agar diffusion technique using *Escherichia coli, Salmonella typhi, Staphylococcus aureus, shigella flexneri, Klebsiella aerogenes and Entero faecalis*. **Results:** All oil extracts possessed antimicrobial activity against most of the bacteria. The clove oil extract showed the highest mean zone of inhibition ranging from 29mm to 35 mm against tested microorganisms, at a concentration of 100 mg/mL. **Conclusions:** The present finding suggests that the fresh carpel oil of *Zanthoxyulum rhetsa* Roxb DC, Dried carpel oil of *Zanthoxyulum rhetsa* Roxb DC, Eucalyptus oil, Clove oil, Palmarose oil and Patchouli oil could be developed as pharmaceutical products.

KEY WORDS: Clove, Eucalyptus, Palmarose, Patchouli, *Zanthoxyulum rhetsa* Roxb DC.

INTRODUCTION

Essential oils of plants and their other products and their secondary metabolites have a great usage in folk medicines as well as in Pharmaceutical industries.

The carpels of Zanthoxyulum rhetsa Roxb DC contained sabinene (66.3%), a-pinene (6.6%), b-pinene (6.3%) and terpinen-4-ol (3.5%) as the major components, the dried fruit contained sabinene (35.7–67.7%) as the major compound (N.B. Shankaracharya et. al 1995, L.J.M. Rao 2000). p-Menthane-1α, 2β, 4β-triol has also been identified (S.K. Paknikar et.al, 1993). The carpels yield an essential oil, which is given in cholera. The seed oil is antiseptic and disinfectant; applied on inflammatory dermatosis. The seed oil is used in dry eczema and dandruff of children in Jointiapur of Sylhet.

Syzygium caryophyllatum (L.) Alston commonly called clove, belonging to Myrtaceae family, is an important aromatic spice. It is commercially cultivated in India, Madagascar, Sri Lanka, Indonesia and the south of China and recently in Bangladesh too on a small scale. Clove oil is widely used for flavouring pastry and in preparation of special sauces .It medicines; it is especially used in the preparations for gum and teeth. Clove bud oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties. (Lee and Shibamoto, 2001; Huang et al., 2002; Velluti et al., 2003). The high levels of eugenol contained in clove essential oil responsible for strong antimicrobial activity.

Pogostemon Desf. is an important genus of family Lamiaceae (K.A. Llamas, 2003). It is commonly called "patchouli" is known for its essential oil. Patchouli oil, though rarely used as dominant source of fragrance, is a basic ingredient of high value perfumes because of its oriental notes and strong fixative properties (A. Kalra et.al, 2006). It is known to possess antifungal properties and is used in skin infections, dandruff and eczema. The oil is also used in aromatherapy for its antidepressant, anti-inflammatory, cytophylactic, deodorant and fungicidal properties (A. Kalra et.al, 2006). The important oil components which are considered for the fragrant and medicinal activities of the oil are patchouli alcohol, α-bulnesene, α- and β-patchoulene (Sarma and T.C. Sarma 2003).

Eucalyptus, a native genus from Australia, belongs to Myrtaceae family. In recent decades, the essential oils and their components of plants have been of great interest as they have been the sources of natural products (Wang W et. al., 2007).The value of *Eucalyptus* oil for medicinal purposes is based largely on the content of a particular oil constituent: 1,8-cineole (cineole or eucalyptol) (Goodger JQD and Woodrow IE (2008). Hot water extracts of dried leaves of *Eucalyptus citriodora* are traditionally used as analgesic, anti-inflammatory and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion (Silva J et.al, 2003), antibacterial (Cimanga K et.al 2002), antifungal (Su YC,

2006), analgesic and anti-inflammatory effects (Silva J et.al, 2003), antioxidative and antiradical (Siramon P et.al, 2007) activities.

Palmarose botanical name is *Cymbopogon martini*. Palmarosa Essential Oil contains potent antifungal properties, making it a popular treatment for Athlete's Foot and other fungal infections. Spider veins may also benefit from an application of Palmarosa. This oil is known to speed the body's regenerative processes, helping all of its systems to work together more efficiently. Emotionally, Palmarosa Oil calms spirals of stressful thoughts. According to the Gas chromatography report, Palmarosa oil constitutes of 14 chemical components that contribute to its fragrance, therapeutic attributes, consistency and quality. Of which, Geraniol contributes to the highest proportion of Palmarosa oil constituents with about 79.4% of its total composition (Schnaubelt, Kurt. 1998).

MATERIAL AND METHODS

Plant Material

Zanthoxyulum rhetsa Roxb DC carpels fresh and dried Oil was obtained by Hydro and stem distillation at kelkar research Sciences at Mulund, Mumbai.

Clove buds were obtained from Indian Council Of agricultural research Andaman Nicobar Islands.

Pogostemon Desf "patchouli"oil and leaves were obtained from Tempo fields where it is cultivated and oil from the Small scale steam-distillation outlet at Itanagar, North East India. Eucalyptus oil and Palmarose oil was obtained from Medicinal and aromatic plants association, Ananad Gujarat, India.

Extraction of essential oil

Zanthoxyulum rhetsa Roxb DC carpels fresh and dried Oil was obtained by Hydro and stem distillation.

Clove buds: The buds were from healthy, well-grown plants. Dried buds (300 g) were grounded in a blender separately. The grounded buds were subjected to hydro distillation using Clevenger apparatus for 4 h for isolation of oils separately (Clevenger, 1928). The oil samples were stored at 0°C in air-tight containers after drying them over anhydrous sodium sulfate and filtered before going to GC-MS analysis.

Pogostemon Desf "patchouli"oil: The fresh leaves biomasses (100 g) of was subjected to hydro distillation by the use of a Clevenger-type apparatus for 8 h.

The leaves of Palmarosa were obtained and oil of Palmarosa was extracted by steam distillation method from the dried grassy leaves that are harvested before flowering at MAPAI, Anand Gujarat, India.

The leaves of Eucalyptus oil of leaves were extracted by steam distillation at MAPAI, Anand Gujarat, India.

Table 1: Different oils used in the study

Sr. No.	Oil
1	**Dried carpels of *Zanthoxyulum rhetsa* Roxb DC**
2	**Fresh carpels of *Zanthoxyulum rhetsa* Roxb DC**
3	**Eucalyptus oil**
4	**Clove oil**
5	**Palmarosa oil**
6	**Patchouli**

Microorganisms Collection

The cultures of *Escherichia coli, Salmonella typhi, Staphylococcus aureus, shigella flexneri, Klebsiella aerogenes and Entero faecalis.* were obtained from Bacteriology department, Haffkine Institute. (Table 2)

Table 2: List of Microorganisms with ATCC/ NTCC and Standards Disc used against Microorganisms

Name of Microorganism (Bacterial)	ATCC/NCTC	Standard
Escherichia coli	ATCC 25922	Azetronam
Salmonella typhi	NCTC 786	Erythromycin
Staphylococcus aureus	ATCC 6538	Amoxyclav
Klebsiella aerogenes	Clinical Isolate	Piperacillin
Shigella flexneri	Clinical Isolate	Ampicillin
Enterococcus faecalis	ATCC 29212	Co- Trimoxazole

Microbial Screening Antimicrobial activities

Six oil extracts were evaluated by the agar well diffusion method (Murray et al.1995) modified by (Olurinola, 1996).

Media Preparation and its sterilization

Agar well diffusion method (Murray et al, 1995) later modified by (Olurinola 1996) antimicrobial susceptibility was tested on solid (agar-agar) media in petri plates. For bacterial assay nutrient agar (NA) (40 gm/L) and for fungus PDA (39 gm/L) was used for developing surface colony growth. The suspension culture for bacterial cell growth was done in Nutrient broth and for fungal cells in PDB % (w/v) PDB (Potato dextrose broth) was taken for evaluation. All the media prepared was then sterilized by autoclaving the media at (121°C) for 20 min.

Agar well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old -broth culture of respective bacteria and fungi. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of each plant extract was prepared at a concentration of 1 mg/ml in different plant extracts viz. Methanol, Ethanol, Petroleum Ether, and Water. About 100 µl of different concentrations of plant solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 hours for bacterial pathogens and 28°C for 48 hours fungal pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded. Test for antimicrobial activity The antibacterial assay was carried out by micro dilution method in order to determine the antibacterial activity of compounds tested against the pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 X 107 CFU/ml. The inocula were prepared and stored at 4^{o} C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. All experiments were performed in duplicate and repeated three times.

RESULTS

The antibacterial activities of the plant volatile oils presented in (Table 3). *Shigella flexneri* and *Escherichia coli* strains demonstrated some degree of sensitivity to the oils tested, although the growth of bacteria were strongly inhibited by Clove oil followed by Eucalyptus

oil, Palmarose oil, Patchouli oil *Zanthoxyulum rhetsa* Roxb DC and Dried carpel oil of *Zanthoxyulum rhetsa* Roxb DC (Fig 1).

Table 3: Antimicrobial activity of different oils

Microorganism	Fresh carpel *Zanthoxyulum rhetsa* Roxb DC	Dried carpel *Zanthoxyulum rhetsa* Roxb DC.	Eucalyptus oil	Clove oil	Palmarose Oil	Patchouli oil
Escherichia coli	Nil	Nil	Nil	32mm	Nil	Nil
Salmonella typhi	18mm	16mm	20mm	32mm	20mm	19mm
Staphylococcus aureus	21mm	16mm	30mm	29mm	23mm	20mm
shigella flexneri	Nil	Nil	Nil	33mm	Nil	Nil
Klebsiella aerogenes	15mm	0	15mm	35mm	19mm	18mm
Entero faecalis	15mm	13mm	24mm	22mm	17mm	15mm

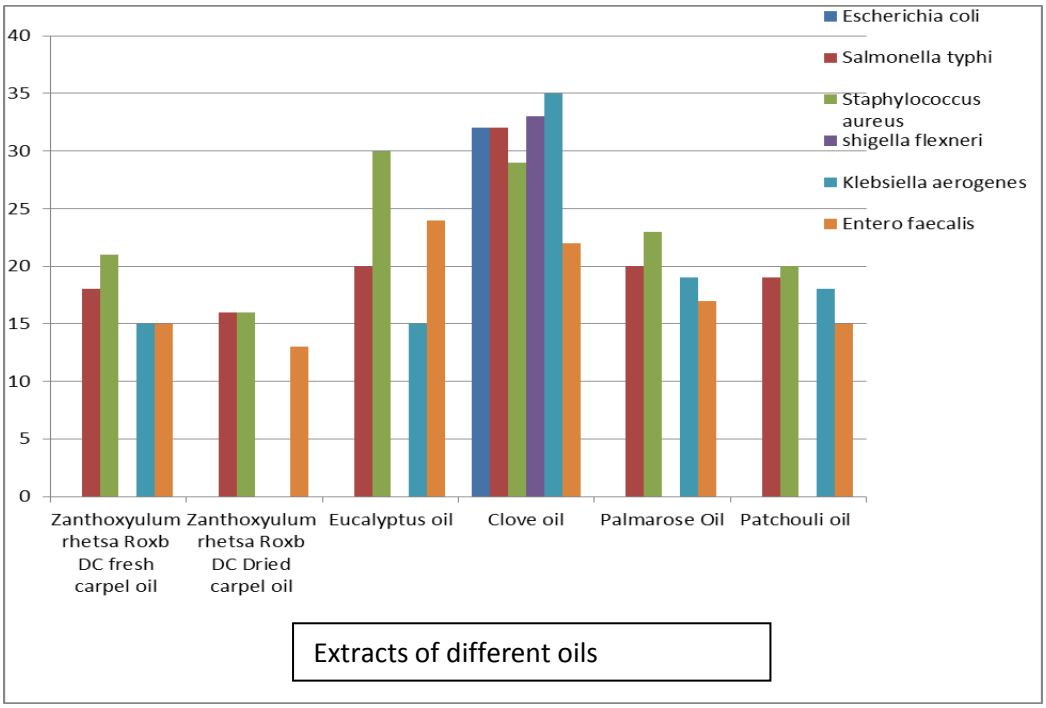

Figure 1: Antimicrobial activity of different oils.

DISCUSSION

The activity of the oils would be expected to relate to the respective composition of the plant volatile oils, the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic interactions between components. As food preservatives, volatile oils may have their greatest potential use. Spices, which are used as integral ingredients in cuisine or added as flavouring agents to foods, are present in

insufficient quantities for their antimicrobial properties to be significant. However, spices are often contaminated with bacterial and fungal spores due to their volatile oil content, often with antimicrobial activity, being enclosed within oil glands and not being released onto the surface of the spice matter. Volatile oils, which often contain the principal aromatic and flavouring components of herbs and spices, if added to foodstuffs, would cause no loss of organoleptic properties, would retard microbial contamination and therefore reduce the onset of spoilage. In addition, small quantities would be required for this effect. Furthermore, evidence suggests that these oils possess strong antioxidant activities. (Dorman, 1999 and Youdim et.al. 1999).

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