

## IN-VITRO ANTI-BACTERIAL AND ANTI-FUNGAL ACTIVITY OF SELECT ESSENTIAL OILS

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Received: 20 May 2014 Revised and Accepted: 19 Jun 2014

### ABSTRACT

**Objective:** Essential oils (E.O's) are highly potent oils, extracted from leaves, flowers, buds, twigs and fruits etc. It has been reported that E.O's have antiseptic and disinfectant activities. The present investigation was undertaken to evaluate the in-vitro anti-bacterial and anti-fungal activity of selected E.O's against few bacterial & fungal strains in combination and alone at increased concentration.

**Methods:** E.O's of orange, palmarosa moha and palmarosa CN-5 were tested for antibacterial and antifungal activity against two human pathogenic bacteria, and four plant pathogenic fungi by disc diffusion method using nutrient agar and potato dextrose agar slants.

**Results:** Palmarosa CN-5 oil was found most effective against *Staphylococcus aureus* whereas Orange oil showed lower activity against tested bacterial strains. Palmarosa moha oil was more potent against *Proteus vulgaris*. All the tested fungi exhibit susceptibility to the selected essential oils with Palmarosa moha oil being the most effective. The minimum bactericidal concentration (MBC) of orange oil, palmarosa moha oil and palmarosa CN-5 oil was ranged from 20-60 µl / ml. These studies led to choose the most effective essential oil.

**Conclusion:** The data obtained from the present investigation indicates that Palmarosa moha and Palmarosa CN-5 oils are most effective in inhibiting the growth of the microorganisms and thus were used for the MBC analysis. The significant differences ( $P < 0.05$ ) among E.O's, suggest that E.O's represents a good alternative to eliminate microorganisms that can be a hazard for human health and can attributing to the synergistic effects of its diverse major and minor components.

**Keywords:** Essential oils (E.O's), Antibacterial activity, Antifungal activity, Minimum bactericidal concentration (MBC), ZONE of inhibition (ZOI).

### INTRODUCTION

Essential oils (E.O's) are highly potent oils, extracted from leaves, flowers, roots, buds, twigs, rhizomes, bark, seeds and fruits etc. Essential oils are found in special secretory glands of plants and are aromatic in nature. These aromatic substances are stored as a byproduct in certain plants because of its metabolism. Each essential oil has its very own blue print that is absolutely unique.

There are around 3000 different essential oils world wide and only 300 essential oils are commonly used. It was believed and later confirmed that plants had anti-bacterial properties [1]. The 'first-aid' kit containing myrrh essential oil was used by few Greek soldiers in the battle field and was introduced by physician Galen. In 1910, it was observed that the essential oil of oregano is the strongest plant derived antiseptic known to date [2]. Oregano is approximately seventy six times more active than isolated phenol on *Bacillus cereus* [2]. The disinfectant power of chaulmoogra essential oil was also compared with that of vegetable oils and animal oil's [3]. The potential of essential oils as antimicrobial agents in perfumes, cosmetics and against wood pathogens was well studied [4]. The essential oil of Lemon and orange was reported to have more antiseptic action than Phenol [5]. It was also observed that addition of orange oil or d-limonene oil increases the preservative properties of sodium benzoate [6]. It was observed that liquid seasoning containing emulsified essential oil have little or no anti-bacterial action because partitioning between oil and aqueous phase reduces the concentration of the antiseptic constituent of the essential oil. Essential oils were also used in food as flavoring agents and are generally accepted by Food and Drug Administration (FDA) as additives in certain type of foods [7-9]. Essential oils, an odorous and volatile product of plant secondary metabolism have a wide use in preservation as well as in fragrance industries. The anti-microbial properties of essential oils have been known for years, recently a large number of essential oils and their constituents have been investigated for their antimicrobial properties against few bacteria and fungi [10]. Essential oils showed antimicrobial activity against a wide range of bacteria including antibiotic resistant species and

fungal species [11-12]. They can affect both Gram positive and Gram negative bacteria in addition to yeasts and filamentous fungi [13-19]. Among such essential oils, the oil of commercial importance is Palmarosa oil, its antifungal action being attributed mainly due to its Geraniol content [20]. Palmarosa oil has high value in the perfumery industry as a source of high grade Geraniol [21]. It exhibits fungistatic action against the filamentous fungi, *Aspergillus niger*, *Chaetomium globosum* and *Penicillium funiculosum* [22] and is considered to provide protection against mosquitoes (*Anopheles culicifacies*) [23]. *Cryptococcus neoformans*, a fungus which causes infections during the last stage of AIDS is inhibited by both Palmarosa oil and Geraniol [24]. In recent years there has been an increasing interest for the use of natural substances because of the safety concern of the synthetic compounds, this encouraged more detailed studies of plant resources [10]. Recently, great attention has been given to food preservation methods and to control the potential pathogens in the animal gastrointestinal tract using healthy natural products as essential oils as an alternative to the antibiotics [25-26], moreover, in clinical studies, the topical use of essential oils is the most promising strategy for the treatment of both skin and mucous membrane [27]. The aim of this work was to determine the in-vitro anti-bacterial and anti-fungal activity against few bacterial & fungal strains in combination and alone in order to establish the time survival kinetics of these micro-organisms when incubated with increased concentration of essential oils.

### MATERIAL AND METHODS

Bacterial strains like *Staphylococcus aureus* (MTCC 1144), *Proteus vulgaris* (MTCC 1771) and fungal strains like *Aspergillus niger* (MTCC 9652), *Penicillium chrysogenum* (MTCC 6477), *Fusarium acuminatum* (MTCC 1983) and *Phanerochaete chrysosporium*, were procured from department of pathology, Mayo hospital, Nagpur. The stock culture was maintained on nutrient agar slants. The culture was incubated at 35-37°C for 24 hours. The stock culture of fungi was maintained on potato dextrose agar (PDA) procured from Himedia laboratories Pvt. Ltd, Mumbai, India and the incubation was processed at 20°C for 07 days.

The antibacterial and antifungal activity was determined by well diffusion method [28-30] and the growth of bacteria and fungi was monitored after exposure to essential oils. The micro-organisms were grown overnight on nutrient agar media and potato dextrose agar media. The day after, bacterial cells (*Staphylococcus aureus* and *Proteus vulgaris*) were inoculated by streaking method on the soft agar medium and about 10 ml of media was poured on Petri-dishes by pour plate method. When the agar was solidified, essential oils were individually impregnated to the sterile whattman chromatographic paper (05 mm) with 20, 30, 40, 50 and 60  $\mu$ l quantities and seeded on the agar plates. The inhibition halos were measured after 24 hours and 48 hours of incubation at different temperatures (37°C temperature for bacteria and 30°C for fungi). Tween-80 was used as negative control. The standard reference antibiotics, Neomycin (30  $\mu$ g/disc, Himedia laboratories Pvt. Ltd., Mumbai, India) and Cloxacillin (10  $\mu$ g/disc, Himedia laboratories

Pvt. Ltd., Mumbai, India) were used as positive control for bacteria and fungi. Each assay was replicated twice. The diameter of clear zone (zone of inhibition) around the disc was measured and expressed in milli meters [31].

## RESULT AND DISCUSSION

Antimicrobial activity of essential oils (orange oil, palmarosa moha oil and Palmarosa CN-5 oil) was assessed by disc diffusion method on human pathogenic bacteria i.e. *Staphylococcus aureus*, *Proteus vulgaris* and plant pathogenic fungi i.e. *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium acuminatum* and *Phanerochaete chrysosporium*. The in-vitro antimicrobial activity of orange oil, Palmarosa moha and Palmarosa CN-5 against the microorganisms employed and its activity potential were quantitatively assessed by presence or absence of zone of inhibition. The result of antimicrobial disc diffusion assay is summarized in table 1.

**Table 1: R, Resistant, Nx: Neomycin, Cx: Cloxacillin**

Microorganism	Essential Oil Zone of inhibition (mm)					Standard Zone of Inhibition (mm)	
	20 $\mu$ l	30 $\mu$ l	40 $\mu$ l	50 $\mu$ l	60 $\mu$ l	Nx	Cx
<b>Orange Oil</b>							
<i>Staphylococcus aureus</i>	5.6	5.6	6.0	6.0	6.0	7.8	R
<i>Proteus vulgaris</i>	6.5	7.0	7.5	8.0	8.5	26.8	29.1
<i>Aspergillus niger</i>	R	R	R	R	R	R	R
<i>Penicillium chrysogenum</i>	R	R	R	R	R	R	R
<i>Fusarium acuminatum</i>	R	R	R	R	R	R	R
<i>Phanerochaete chrysosporium</i>	R	R	R	R	R	R	R
<b>Palmarosa Moha Oil</b>							
<i>Staphylococcus aureus</i>	8.0	8.0	8.5	9.0	9.0	7.8	R
<i>Proteus vulgaris</i>	11.0	13.0	16.0	17.0	19.0	26.8	29.1
<i>Aspergillus niger</i>	20.0	20.5	21.0	21.5	22.0	R	R
<i>Penicillium chrysogenum</i>	15.0	15.5	16.0	16.0	16.0	R	R
<i>Fusarium acuminatum</i>	20.0	22.0	24.0	26.0	26.0	R	R
<i>Phanerochaete chrysosporium</i>	11.0	11.5	12.0	12.5	13.0	R	R
<b>Palmarosa CN-5 Oil</b>							
<i>Staphylococcus aureus</i>	9.5	9.5	10.0	10.0	10.0	7.8	R
<i>Proteus vulgaris</i>	11.0	11.0	12.0	12.5	13.0	26.8	29.1
<i>Aspergillus niger</i>	16.0	16.5	17.0	17.5	18.0	R	R
<i>Penicillium chrysogenum</i>	17.0	17.5	18.0	18.5	19.0	R	R
<i>Fusarium acuminatum</i>	16.0	16.5	17.0	17.5	18.0	R	R
<i>Phanerochaete chrysosporium</i>	12.0	13.0	13.0	13.0	13.0	R	R

According to the results illustrated in table-1, the zone of inhibition for *Staphylococcus aureus* was measured 5.6 mm at 20 & 30  $\mu$ l while it was 6.0 mm, when the bacteria was exposed to 40, 50 & 60  $\mu$ l of orange oil. The antibacterial activity of orange oil against *Staphylococcus aureus* presented in (Fig.1)

In all the experiments carried out with test organism *Staphylococcus aureus*, the ZOI varied from 5.6 to 6.0 mm with an average of 5.84 mm. It has been observed that *Staphylococcus aureus* is one of most susceptible bacteria to plant extracts [27-30]. *Staphylococcus aureus* exhibits resistance to antibiotic Cloxacillin which was taken as positive control. The ZOI against *Proteus vulgaris* was observed 6.5, 7.0, 7.5, 8.0 & 8.5 mm for 20, 30, 40, 50 & 60  $\mu$ l of orange oil respectively (Fig.3).

The antibacterial activity of orange oil was found lower than that of Neomycin (ZOI 26.5 mm) and Cloxacillin (ZOI 28.5 mm) (Fig.4). Plant pathogenic fungi (i.e. *Aspergillus*, *Penicillium*, *Fusarium* and *Phanerochaete*) were found resistant to orange oil. The antibacterial and antifungal activity of orange oil was insignificant ( $P > 0.05$ ) as compared to standard antibiotics.

The ZOI against *S. aureus* was observed as 8.0, 8.0, 8.5, 9.0 & 9.0 mm for 20, 30, 40, 50, & 60  $\mu$ l of Palmarosa moha oil respectively (Fig.1). The activity of Palmarosa moha oil was found better than that of Neomycin (ZOI 7.5 mm) and orange oil. (Fig.5)

P. CN-5 oil was found to most active antibacterial agent against *Staphylococcus aureus* among the tested E.O's (Fig.6).

The antibacterial activity of Palmarosa moha oil was observed to increase gradually with increase in concentration against *Proteus*

*vulgaris* (Fig.3) but it was lower than that of positive control Neomycin (ZOI 26.5 mm) and Cloxacillin (ZOI 28.5 mm) (Fig.7)

The antibacterial activity of Palmarosa moha oil was found higher than that of orange oil and Palmarosa CN-5 oil (Fig.8). The Zone of inhibition varied from 11.0 – 19.0 mm with an average of 15.2 mm.

Palmarosa moha oil exhibited strong antifungal activity against all tested fungal species and also exhibited maximum antifungal activity amongst the selected essential oils (Fig.9).

Among the tested fungi, *Aspergillus niger* was most susceptible to Palmarosa moha oil, the antibacterial and antifungal activity of Palmarosa moha oil was significant ( $P < 0.05$ ) than that of Palmarosa CN-5 oil and standard antibiotics (Fig.10).

The ZOI against *Staphylococcus aureus* was observed 9.5, 9.5, 9.5, 10 & 10 mm for 20, 30, 40, 50 & 60  $\mu$ l of Palmarosa CN-5 oil respectively (Fig.1). The antibacterial activity of P. CN-5 oil was found better than that of positive control i.e. Neomycin (ZOI 7.5 mm) (Fig.11).

The antibacterial activity of P. CN-5 oil was observed to increase gradually with increase in concentration against *Proteus vulgaris* (Fig.12) but it was lower than that of positive control i.e. Neomycin (ZOI 26.5 mm) & Cloxacillin (ZOI 28.5 mm) and was in between the orange oil and P. moha oil (Fig.8).

P. CN-5 oil exhibited good antifungal activity against all the tested fungal species but was less effective than that of P. moha oil (Fig.13). Among the tested fungi, *Penicillium chrysogenum* was not sensitive to P. CN-5 oil. The antibacterial and antifungal activity of P. CN-5 oil was found to significant ( $P < 0.05$ ) than that of orange oil but was less significant than P. moha oil.

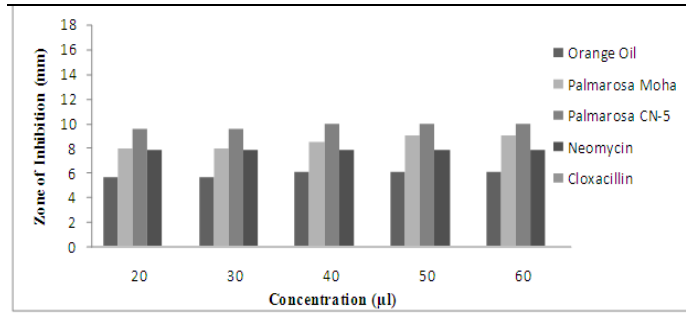


Fig. 1: Antibacterial activity of essential oils on Gm +ve Staphylococcus aureus by Disc diffusion method and was found lower than that of Neomycin (ZOI: 7.5 mm) which was taken as positive control (Fig.2).

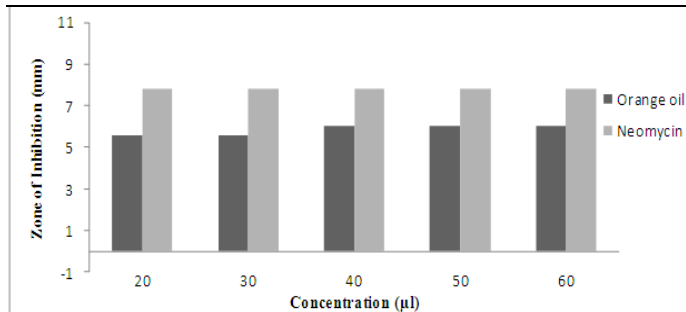


Fig. 2: Antibacterial effect of Orange oil and Neomycin on Staphylococcus aureus

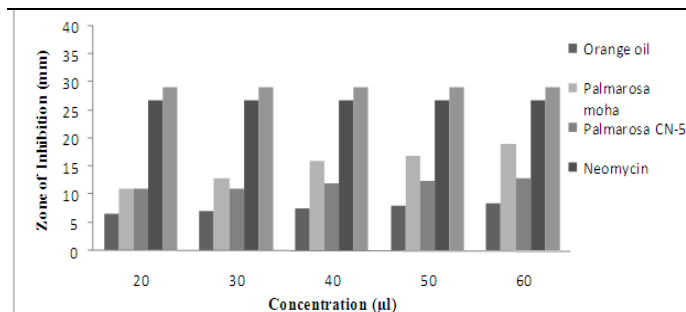


Fig. 3: Antibacterial activity of essential oils on Gram-ve Proteus vulgaris by Disc diffusion method

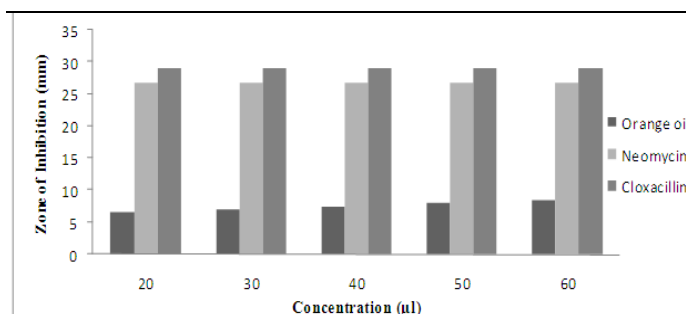


Fig. 4: Antibacterial effect of Orange oil, Neomycin and Cloxacillin on Proteus vulgaris

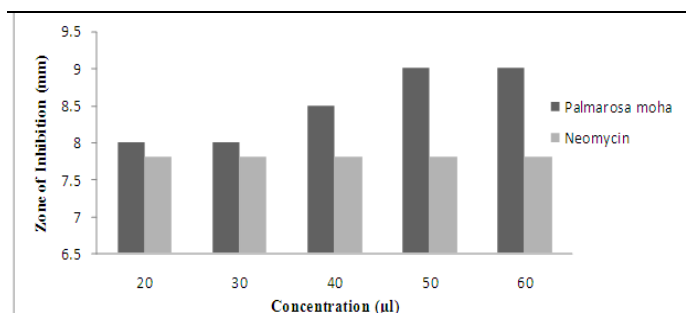


Fig. 5: Antibacterial effect of Palmarosa moha oil and Neomycin on Staphylococcus aureus

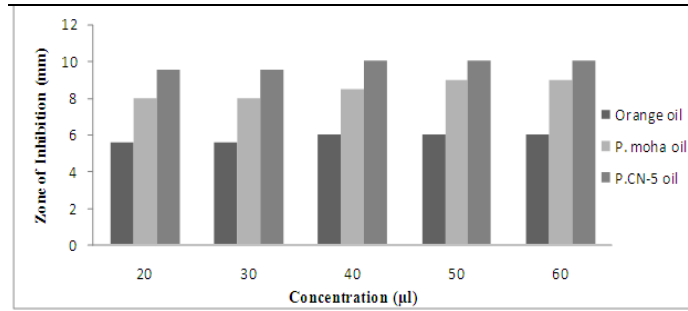


Fig. 6: Antibacterial effect of Orange oil, P. moha oil and P. CN-5 oil on Staphylococcus aureus

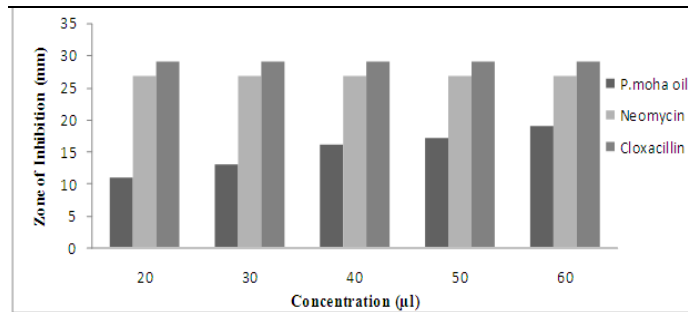


Fig. 7: Antibacterial effect of P. moha oil, Neomycin and Cloxacillin on Proteus vulgaris

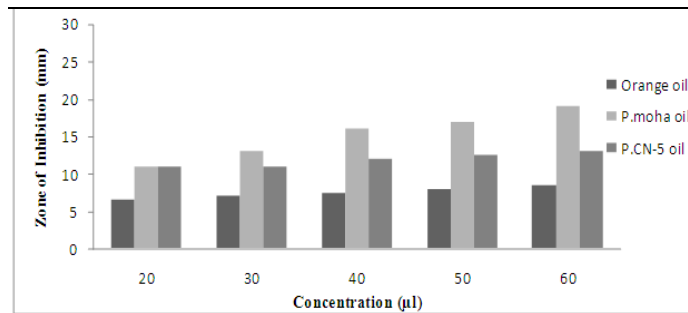


Fig. 8: Antibacterial effect of Orange oil, P. moha oil and P. CN-5 oil on Proteus vulgaris

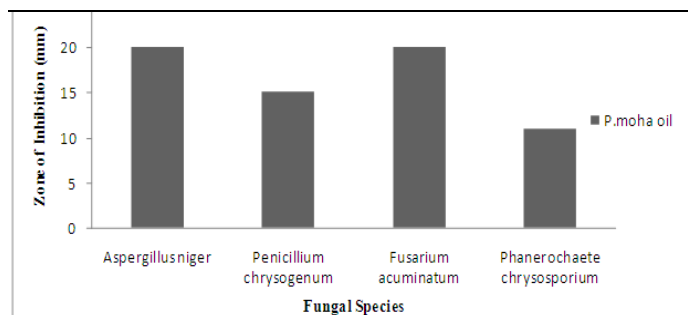


Fig. 9: Antifungal activity of P. moha oil

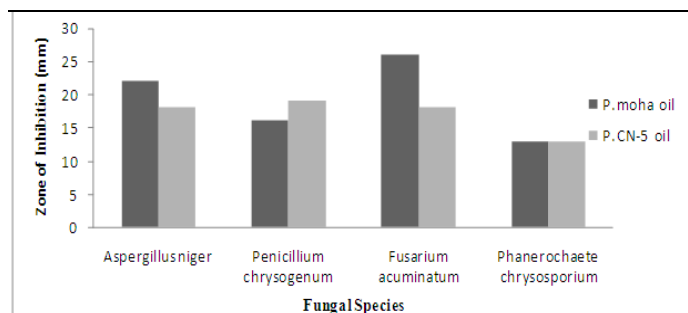


Fig. 10: Antifungal activity of essential oils by Disc diffusion method

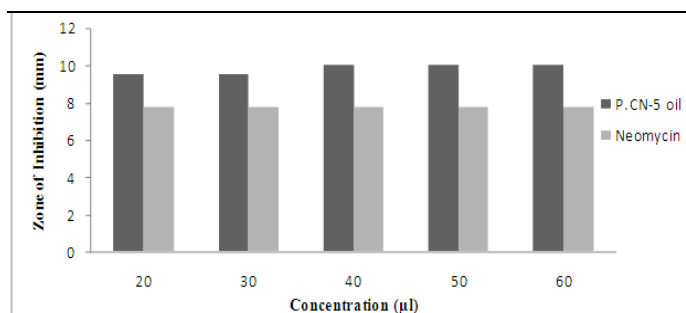


Fig. 11: Antibacterial effect of P. CN-5 oil and Neomycin on *Staphylococcus aureus*

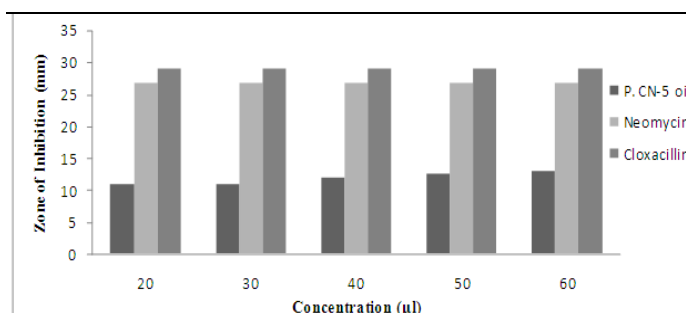


Fig. 12: Antibacterial effect of P. CN-5 oil, Neomycin and Cloxacillin on *Proteus vulgaris*

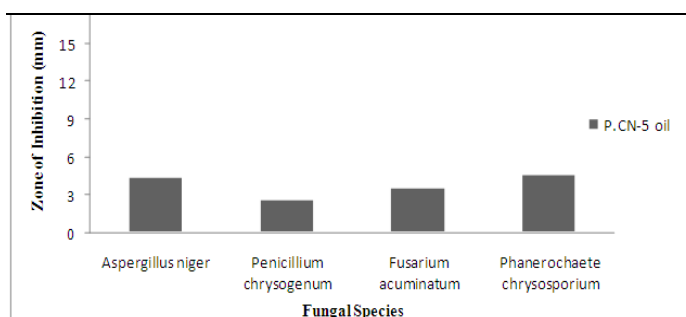


Fig. 13: Antifungal activity of P. CN-5 oil

## CONCLUSION

The selected essential oils (Orange oil, Palmarosa moha oil & Palmarosa CN-5 oil) exhibited good antibacterial activity against all the tested organisms (*Staphylococcus aureus* & *Proteus vulgaris*) that are known human pathogens. Palmarosa CN-5 oil was observed to show maximum activity against *Proteus vulgaris* and *Staphylococcus aureus* strains whereas Orange oil showed lesser activity against both the bacterial species. Palmarosa moha oil was found to be more potent against *Proteus vulgaris*. All the tested fungi were observed to have susceptibility to all the selected essential oils with P. moha oil being the most effective antifungal E.O.

## FUTURE RESEARCH NEEDS:

The information available to evaluate the anti microbial activity of different E.O's with reference to bacteria and fungi are inadequate and there is an ample scope, as delineated here under, to generate the data in this regard.

✓ E.O's can be used as ingredients of Natural drugs and natural pesticides.

✓ Evaluation of toxicological studies of E. O's on animals and plants

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