ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST TEST ORGANISMS

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ABSTRACTS:

Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties.Some oils have been used in treatment of infection in gallbladder**.** Essential oils are a rich source of biologically active compounds. Therefore, it is reasonable to expect a variety of plant essential oils with specific as well as general antimicrobial activity **.** The activity of essential oils was evaluated against five bacterial pathogens including *E. coli, Salmonella sp, Klebsiella, Streptococcus, Staphylococcus* (test bacteria) using disc diffusion method. Microorganisms used in this study were isolated from patients of GB diseases. Test bacteria were characterized on the basis of their biochemical characteristics. Screening of essential oils for antimicrobial activity was performed by disc diffusion method, which is normally used as rapid method to assess antimicrobial activity of essential oils **.** Our study have analyzed that these essential oils are useful for the preparation of herbal drugs which can be used in the treatment of infective diseases. It can also be concluded that essential oils shows good activity against test bacteria.

KEY WORDS: Essential oils, Disc diffusion method, antimicrobial activity, Inhibition zone.

INTRODUCTION:

The antimicrobial activity of garlic, cinnamon, and cloves has been studied since the end of last century, and the active components in these herbs are known *(1).* Antimicrobial activity of plant extracts is frequently due to the essential oil fraction, or to sulfur-containing compounds in the aqueous phase *(2).*Essential oils have been used medicinally in past. Medical applications proposed by those who sell essential oils range from skin treatments to remedies for gallbladder diseases and often are based solely on historical accounts of use of essential oils for these purposes. The antimicrobial activity of Essential oils (EOs) has long been recognised and they have been extensively tested *in vitro* against a wide range of pathogenic bacteria and fungi (3). Some oils have been used in gallbladder cancer treatment.(4)

The mechanism by which the essential oils exert their antimicrobial activity is poorly understood but the main target appears to be the cell membrane of bacterial cells (5).Essential oils such as cinnamon, thyme,eucalyptus, clove and geranium have been traditionally used by people for various years.

MATERIALS AND METHODS:

Essential oils

Essential oils obtained from department of pharmaceutical science, Jiwaji University, Gwalior, India were used in this study. These oils were selected on the basis of literature survey and their use in traditional and conventional medicine.

List of selected essential oils used in the study.

Code	Common name	Botanical name	Family
EO-1	Rasna Oil	*Pluchea lanceolata*	Compositae
EO-2	Ajwain Oil	*Carum copticum*	Umbelliferae
EO-3	Tulsi Oil	*Ocimum sanctum*	Labiateae
EO-4	Peppermint Oil	*Mentha piperata*	Alliaceae
EO-5	Garlic Oil	*Allium sativum L.*	Alliaceae
EO-6	Til Oil	*Tagetes minuta L.*	Alliaceae
EO-7	Cinnamomum Oil	*Cinnamomum zeylanicum*	Lauraceae
EO-8	Turpentine Oil	*Pinus pinaster*	Pinaceae
EO-9	Eucalyptus Oil	*Eucalyptus globulus*	Pinaceae
EO-10	Clove Oil	*Syzygium aromaticum*	Myrtaceae
EO-11	Mentha Oil	*Mentha arvensis*	Lamiaceae
EO-12	Olive Oil	*Olea europea*	Oleaceae
EO-13	Masturd Oil	*Brassica campestris*	Poaceae
EO-14	Caster Oil	*Ricinus communis*	Euphorbiaceae
EO-15	Coconut Oil	*Cocus nucifera*	Pamae

Microorganisms used in this study were isolated from patients of GB diseases. Test bacteria were characterized on the basis of their biochemical characteristics. The bacteria used in present study were *E.coli, Klebsiella sp, Salmonella sp, Streptococcus sp* and *Staphylococcus sp.* The cultures of bacteria were maintained in nutrient agar slants at 4°C throughout the study and used as stock culture.

Antimicrobial analysis of essential oils

Screening of essential oils for antimicrobial activity was performed by disc diffusion method, which is normally used as rapid method to assess antimicrobial activity of essential oil**.** Antimicrobial susceptibility analysis was performed over Mueller Hinton Agar (HiMedia), which is a only susceptibility medium validated by NCCLS (National committee for clinical laboratory standards). The media was prepared as indicated in previously.

Inoculums preparation and Inoculation Procedure

Inoculums of *0.5* McFarland standards was prepared according to previously mentioned method and within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab was dipped into the suspension. Pressed firmly against the inside wall of the tube just above the fluid level and rotated to remove excess liquid. The swab was streaked over the entire surface of the medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums. Finally swab was streaked all around the edge of the agar surface.

Antimicrobial disks

The antimicrobial disks were prepared from Whatman's filter paper no. 1. These discs were sterilized in hot air oven and stored in the refrigerator *(4°C)* for use. Upon removal of the discs from the refrigerator, the package containing the cartridges was left unopened at room temperature for approximately 1 hour to allow the temperature to equilibrate. This reduces the amount of condensation on the discs. Under aseptic condition empty sterilized discs were impregnated with 25 µL of respective essential oil and placed on agar surface . Antimicrobial disks were applied to the plates within 15 minutes after inoculation. Discs were placed individually with sterile forceps and then gently, pressed down onto the agar, Diffusion of the essential oil in the disc begins immediately; there fore, once a disc contacts the agar surface, the discs were not moved.

Recording and interpreting results

After the inoculation of bacterial culture and keeping the discs over media, plate was incubated at 37^0C for 16 to 18 hours. After incubation, the diameter of the zones of complete inhibition was measured and recorded in millimeters. The measurements were made with the help of antibiotic zone scale **(Hi-Media)** on the

undersurface of the plate without opening the lid. Streaked, well-punched Mueller Hinto agar plate (without essential oils) is used as a control for the comparition of activity of herbal antimicrobial compounds.

RESULTS:

After testing the antimicrobial activity of herbal plant extracts, antimicrobial activity of essential oils against test bacteria is performed. 15 types of essential oils are used, among these oils Eukalyptus (*Eukalyptus globules*), Dalchini, Clove (*Eugenia caryophyllatus)* and Mentha (*Mentha piperita*) shows highest activity (strong inhibition zone on MH agar) against almost test organisms but Til (*Sesamum indicum*), Olive (*Olea europaea*), Mustard (*Brassica campestris*), Caster (*Ricinus communis*) and Coconut oil (*Cocos nucifera*) shows no activity (no zone forms around these essential oils) against test bacteria. Tulsi (*Ocimum sp*.), Piperment (*Mentha piperita*) and Rasna (*Pluchea lanceolata*) shows least activity against test bacteria (weak inhibition zone form). Generally, many Indian people use Dalchini, Clove and Mentha in there own kitchen very frequently. Our study have analyzed that these essential oils are useful for the preparation of herbal drugs which can be used in the treatment of infective diseases. It can also be concluded that essential oils shows good activity in comparison to plant extract

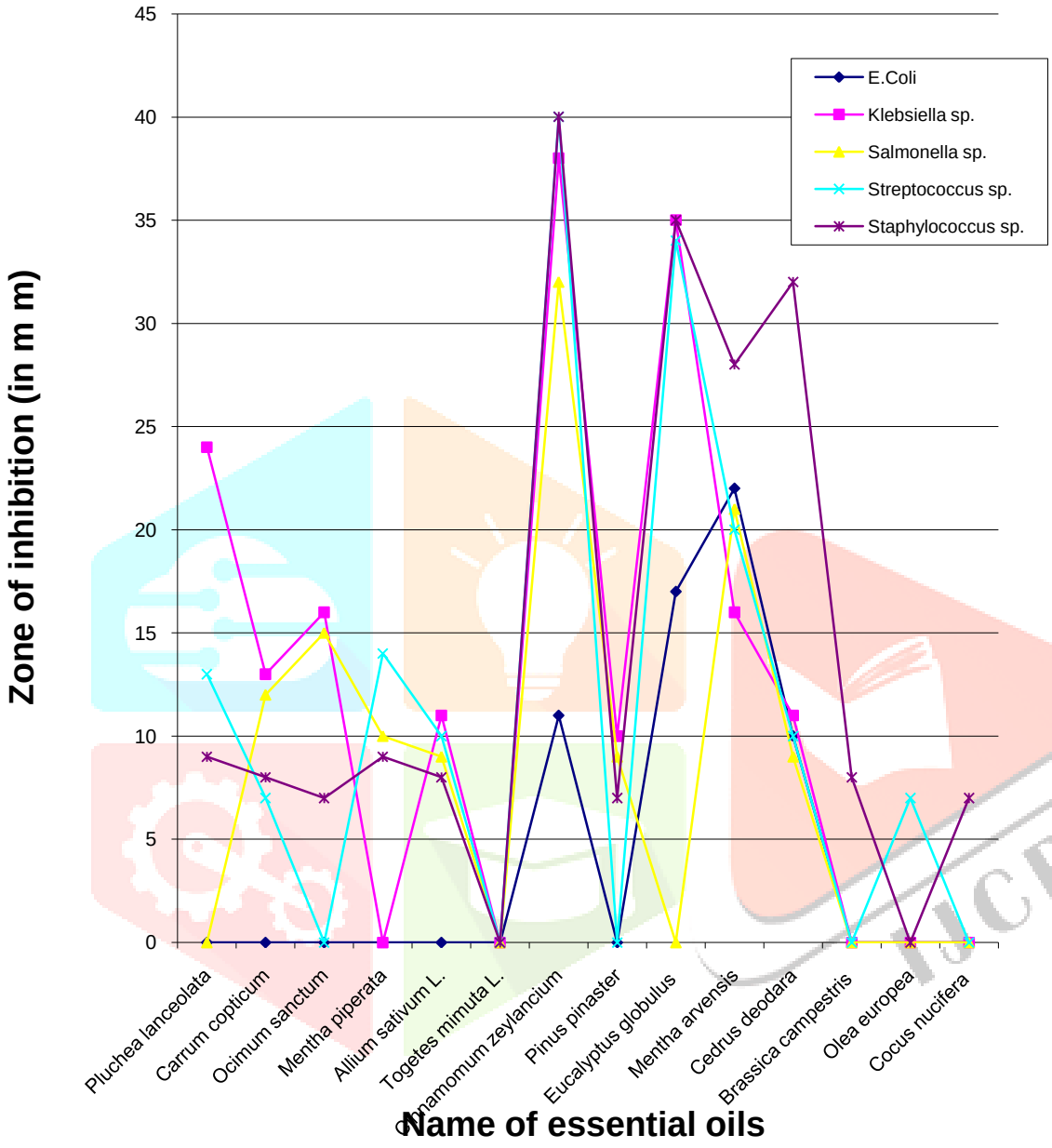
Table :Inhibition zone diameter of essential oils against bacteria

S.no.	Essential oils	E.coli	Klebsiella sp.
1	*Pluchea lanceolata*	0	24 m.m.
2	*Carum copticum*	0	13 m.m.
3	*Ocimum sanctum*	0	16 m.m.
4	*Mentha piperata*	0	0
5	*Allium sativum L.*	0	11 m.m.
6	*Tagetes minuta L.*	0	0
7	*Cinnamomum zeylanicum*	11 m.m.	38 m.m.
8	*Pinus pinaster*	0	10 m.m.
9	*Eucalyptus globulus*	17 m.m.	35 m.m.
10	*Syzygium aromaticum*	22 m.m.	16 m.m.
11	*Mentha arvensis*	10 m.m.	11 m.m.
12	*Cedrus deodara*	0	0
13	*Brassica campestris*	0	0
14	*Olea europea*	0	0
15	*Cocus nucifera*	0	0

R=Resistent (<10 mm)

Salmonella	Streptococcus	Staphylococcus
0	13 m.m.	R
12 m.m.	R	R
15 m.m.	0	R
10 m.m.	14 m.m.	R
R	10 m.m.	R
0	0	0
32 m.m.	40 m.m.	40 m.m.
R	0	R
0	34 m.m.	35 m.m.
21 m.m.	20 m.m.	28 m.m.
R	10 m.m.	32 m.m.
0	0	R
0	R	0
0	0	R
0	0	0

Efficacy of essential oils against bacteria



DISCUSSION:

Anecdotal evidence and the traditional use of plants as medicines provide the basis for indicating which essential oils may be useful for specific medical conditions. Historically, many plant oils such as tea tree, tulsi and clove, have been used as topical antiseptics, or have been reported to have antimicrobial properties **(6).** It is important to investigate scientifically those plants, which have been used in traditional medicines as potential sources of novel antimicrobial compounds **(7).** Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required. Various publications have documented the antimicrobial activity of essential oils including rosemary, peppermint, Ajwain, basil, tea tree, til, terpentine, mentha and fennel **(8).** Oils such as olive, caster and coconut were shown to possess little or no antimicrobial activity **(9).** Of these, only cinnamomum oil showed any significant antimicrobial activity. Not surprisingly, the fixed oils, which are used largely as diluents for essential oils or as sources of dietary fatty acids **(10),** did not show significant antimicrobial activity.

When comparing data obtained in different studies, most publications provide generalizations about whether or not plant oil possesses activity against Gram-positive and Gram-negative bacteria. However, not all provide details about the extent or spectrum of this activity. Some publications also show the relative activity of plant oils and extracts by comparing results from different oils tested against the same organism(s).

The need for a standard, reproducible method for assessing oils has been stressed by several authors **(11).** In view of this, many methods have been developed specifically for determining the antimicrobial activity of essential oils **(12).** The benefits of basing new methods on preexisting, conventional assays such as the NCCLS methods are that these assays tend to be more readily accepted by regulatory bodies **(13).** Also, these methods have been designed specifically for assessing the activity of antimicrobial compounds, and factors affecting reproducibility have been sufficiently investigated. Although NCCLS methods have been developed for assessing conventional antimicrobial agents such as antibiotics, with minor modifications these methods can be made suitable for the testing of essential oils **(14).** For some plant oils, such as wintergreen, eucalyptus, clove and sage, there has been much research and reporting of toxic and irritant properties **(15).**

Several studies **(16)** have shown that cinnamon, clove and rosemary oils had strong and consistent inhibitory effects against various pathogens. Even though earlier studies have reported better antimicrobial activity for eucalyptus oil **(17)** our study showed least inhibitory activity of pinus sp. Among all oils analyzed in this work, the essential oil of cinnamon was the most effective as an antibacterial agent. The antibacterial activity has been attributed to the presence of some active constituents in the oils. Cinnamaldehyde was completely inhibiting

both sensitive and resistant stain of *Helicobacter pylori* **(18).** Cinnamon oil was not harmful when consumed in food products and it inhibited the growth of molds, yeast and bacteria **(19).** This oil is also used in the treatment of cancer and other microbial diseases **(20).**

In current study, Eukalyptus (*Eukalyptus globules*), Dalchini, Clove (*Eugenia caryophyllatus)* and Mentha (*Mentha piperita*) shows highest activity (strong inhibition zone on MH agar) against almost tests organisms but Til (*Sesamum indicum*), Olive (*Olea europaea*), Mustard (*Brassica campestris*), Caster (*Ricinus communis*) and Coconut oil (*Cocos nucifera*) show no activity (no zone forms around these essential oils) against test bacteria. Tulsi (*Ocimum sp*.), Piperment (*Mentha piperita*) and Rasna (*Pluchea lanceolata*) shows least activity against test bacteria (weak inhibition zone form).

CONCLUSION:

Essential oils shows good activity,so theseoils can be uses in diet of gallbladder patients. Present study defined essential oil of *Cinnamomum zeylanicum* was more active against all test bacteria. **)**.In our study tulsi oil showed good activity against *Klebsiella sp* and *Salmonella sp.*

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