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In vitro Antimicrobial and Antifungal Activity of *Mentha piperita* Active Phytoconstituents

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Abstract

Previously quantified extract of *Mentha piperita* species for active phytoconstituents *menthol* and *pulegone* has been evaluated for antibacterial and antifungal activity. For the research study activity on gram positive bacteria *S. Aureus*, gram negative bacteria *E. Coli* and fungal species *C. albicans* were studies. Qualitative and quantitative measurement of active constituents namely pulegone and menthol was previously done with modern analytical techniques. Quantified standards employed for quantification of main constituents in extract. The proposed research article is design to develop novel formulation without added synthetic preservatives. The quantified extract is prepared in series of concentration (10, 15, 20, 25, 30 ppm solution in ethanol). Each concentration is applied to different bacterial and fungal species in sterilized conditions. By the use of disc diffusion method and broth micro dilution method experiments were done. The Zone of Inhibition (mm) values of extract were found in linearity range. Notably, extract of *Mentha piperita* shows remarkable antimicrobial and antifungal activities against the tested clinical pathogens. The results indicate that these active constituents Pulegone and *Menthol* of extract can be used in formulation with replacement of synthetic added preservatives.

***Key words*:** Medicinal Plants, Quantification, Phytoconstituents, Natural Preservatives, Antibacterial, Antifungal

It is very important to do the research on medicinal herbs that have been used in traditional medicines as potential sources of new antioxidants and antimicrobials [1]. Traditionally, many plant oils such as essential oils and extracts have been used to report for possessing antimicrobial properties [2-3]. As well as, the improvement of interest in natural therapies and increased consumer demand for safe, effective and natural products mean that quantitative data on plant oils and their extraction are required [4].

Natural products like active ingredients isolated or separated from plants or herbal preparations with their potent medical use are a relevant group of substances for the food and pharmaceutical industry [5-7]. Nowadays, the modern drugs are also obtained from natural sources, and they are having much demand in market over chemically synthesized drugs. Therefore, drugs obtained from natural sources holding great potential to provide substances of pharmaceutical, food and cosmetic interest [8-10]. Herbal plant medicines emerged as containing highest antioxidant and antimicrobial products as preservatives [11-12]. Antioxidant and antimicrobials added to pharmaceutical formulations offer protection to other oxygen sensitive drug and from microbial growth for example Tulsi, Brahmi, Shatavari etc.

MATERIALS AND METHODS

Sources of test organisms for MIC

To evaluate antimicrobial and antifungal activity different species of bacteria and fungus were chosen like *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* for gram negative bacteria Maconkey agar used. Bacterial growth also checked using Mc-farland reader device for ideal count of microorganism. All material and apparatus used in method were previously sterilized with autoclave.

Preparation of different concentration samples

Ethanolic extract quantified using standard pulegone and menthol by HPTLC and GC method. Prepare series of concentration of extract in ethanol as follows:

Concentration (ppm)	Extract (mg)	Dilution	Volume (ml)	Diffusion (µl)
10000	100	Ethanol	10	2
15000	150	Ethanol	10	2
20000	200	Ethanol	10	2
25000	250	Ethanol	10	2
30000	300	Ethanol	10	2

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Preparation of sample solution

The sample solution of ethanolic extract which was purified by filtration and steam distillation at laboratory scale. That extract was quantified for pulegone and menthol concentration using standard R(+)-pulegone and menthol solid crystals. Sample solutions were prepared as ppm solution in different concentrations such as 10, 15, 20, 25, 30 thousand ppm in ethanol. Ethanol kept as blank.

Preparation of bacterial culture

All media has been prepared as 24-hour old culture. For gram negative bacteria use lactose fermenting colony. Confirmation of growth of colonies was done by IMVIC test. Pink color of bacterial colonies was seen. Extract part of colonies and take reading for ideal count 1×10^8 CFU on McFarland reader. Check sterility, fertility and also other factors like water to affect culture. Maintain pH neutral of media. All bacterial cultures were prepared to McFarland 0.5 standard equivalent to 1×10^8cfu/ml. The bacterial suspension's turbidity was adjusted to McFarland reader.

Procedure: Procedure for disk diffusion assay

The evaluation of ethanolic extract of *Mentha piperita* for antibacterial and antifungal activity was done by the disc diffusion method. Total three strains were used for study which were obtained from microbial culture at Aarman Microlab, Nashik, (Maharashtra) India. Sterile disc moistened with ethanol as control sample and placed on the seeded petri plate. Standard discs of antibiotic and antifungal were used as reference control. All the Petri plates were labelled and sealed. The plates were allowed to diffuse extract for 30 minutes at room temperature and then they were incubated at 37 °C for 24 h. After the incubation period, the zone of inhibition was measured with help of vernier caliper. All the concentrations of extracts were tested in a triplicate manner against each bacterial and fungal species [13-15].

RESULTS AND DISCUSSION

Antibacterial and antifungal sensitivity test of ethanolic extract of *Mentha piperita* was taken in triplicate manner on *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* [16-24]. The zone of inhibition in mm was observed as follows:

Fig 1 Zone of inhibition in mm on gram positive bacteria *Staphylococcus aureus*

Fig 2 Zone of inhibition in mm on gram negative bacteria *Escherichia Coli*

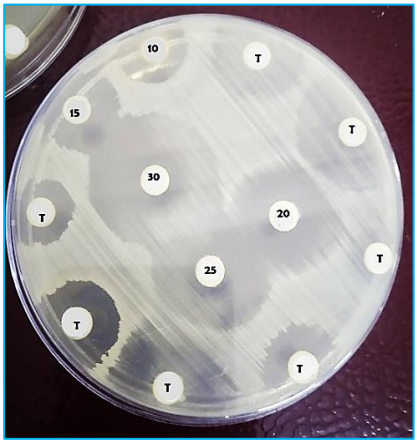

Fig 3 Zone of inhibition in mm on fungus *Candida Albicans*

Table 1 Zone of inhibition (mm) on gram-positive, gram-negative bacteria and fungus on first trial of purified extract of *Mentha piperita*

Bacterium	Zone of inhibition (mm) of different concentration in thousand ppm solution				
	10	15	20	25	30
Staphylococcus aureus	7±1	11±1	14±1	17±1	19±1
Escherichia coli	6±1	10±1	13±1	18±1	20±1
Candida albicans	8±1	13±1	17±1	20±1	22±1

Table 2 Zone of inhibition (mm) on gram-positive, gram-negative bacteria and fungus on second trial of purified extract of *Mentha piperita*

Bacterium	Zone of inhibition (mm) of different concentration in thousand ppm solution				
	10	15	20	25	30
Staphylococcus aureus	7±1	10±1	13±1	17±1	20±1
Escherichia coli	7±1	10±1	13±1	18±1	20±1
Candida albicans	9±1	12±1	17±1	20±1	23±1

Table 3 Zone of inhibition (mm) on gram-positive, gram-negative bacteria and fungus on third trial of purified extract of *Mentha piperita*

Bacterium	Zone of inhibition (mm) of different concentration in thousand ppm solution				
	10	15	20	25	30
Staphylococcus aureus	7±1	11±1	14±1	17±1	19±1
Escherichia coli	6±1	10±1	14±1	18±1	20±1
Candida albicans	8±1	13±1	18±1	21±1	23±1

CONCLUSION

After evaluation of antibacterial and antifungal activity the present study conclude that, the active constituents of extract *pulegone* and *menthol* possess great potential of antibacterial and antifungal action on gram positive and gram-negative bacteria and on fungus as well. The validation study performs on stated species in triplicate manner which shows accuracy results in all trials. There are many types of natural sources that are still not being studied to be used as food preservatives. The presence of active compounds like, flavonoids, quinone, tannins, essential oils etc. in the natural sources will act as the antimicrobial and antioxidant agents. In conclusion, this particular issue provides to the readers the chance to be aware on the recent advancements and development related to the antimicrobial and antioxidant properties of natural substances from different sources that are of interest for the present-day food and pharmaceutical industry. Thus, the development of formulation using extract with active constituent is needed because of natural antimicrobial activity with more effective, selective and less toxic than the synthetic antimicrobial preservatives.

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