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EVALUATION OF ANTI-BACTERIAL ACTIVITY OF ETHANOL AND ACETONE EXTRACTS OF *MENTHA AQUATICA* LEAF

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ABSTRACT

The aim of the project work is to extract the active compounds from leaves of the *Mentha aquatica* by using ethanol and acetone and to compare their anti-bacterial activity. As the synthetic drugs are potentially toxic and are not free of side effects on the host, an attempt has been made to study the anti-bacterial activity by using the extracted crude compounds from *Mentha aquatica* naturally occurring plants. The results showed that ethanol and acetone extracts of leaves of *Mentha aquatica* presented a better inhibitory effect on the test organisms. *Escherichia coli* NCIM 2563 and *Bacillus subtilis*, NCIM 2063 were most susceptible when compared to *Staphylococcus aureus* NCIM 5021 and *Pseudomonas aeruginosa* NCIM 5029. The active substance causing the inhibitory effect which could have been higher in the leaves. The use of acetone as extracting solvents proved to be more efficient in extracting the active compounds than ethanol. Present investigation reveals that *Mentha aquatica* can be used as an alternative to anti-bacterial agent, since it is equally effective against the gram positive and gram negative bacteria. So it is significant to exploit novel anti-bacterial drugs from these medicinal plants.

Key words: *Mentha aquatica*, Anti-bacterial activity, Agar well diffusion assay, ethanol, acetone.

INTRODUCTION

Traditional system of medicine whether they are of Indian, Chines, Tibetan or Thai origins have evolved over several hundreds of thousands of year through transfer of knowledge, and usage practice from generation to generation. (Gomes AT *et al*., 2003; Hamburger MO *et al*., 1987) From the vast array of the Material medica of indigenous system it is thought that investigation and research on medicinal plant it might bring to the scientific world many useful remedies for the alleviation of human suffering. In spite of the remarkable achievement of the modern medicine and medical research these ancient systems continue used to be a major component, effectively used in the control or alleviation of disease (Nickavar B *et al*., 2008; Dambrauskienë E *et al*., 2008)

Plants and plant-based are less toxic and have acceptable side effects.

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It is therefore essential to bring the use of these remedies into an existing frame work of rational scientific use of medicine based on the strong traditional knowledge. Medicinal plants are an important therapeutic aid for various ailments (Nickavar B *et al*., 2008). Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants (Kähkönen MP et al., 2004; Szőllősi R *et al*., 2001). This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants (Capecka E *et al*., 2005)

MATERIAL AND METHODS

PLANT COLLECTION

Plants was collected from Indira Park, Public gardens, Nampally, Hyderabad. The identification was confirmed by Head Department of Botany, Osmania University. The fresh leaves were separated from the plant and washed thoroughly for 2-3 times with running tap water and then with sterile water followed by shade drying. The separated leaves were powdered in a mixer and fine powder was collected by passing through sieve no: 40. The fine powder is used for extraction.

Chemical Collection

Acetones, DMSO, Distilled water are the chemicals collected from UNIQUE CHEMICALS Pvt. ltd., Tilak road, Abids, Hyderabad.

Identification of the Plant

The plant was identified based on the leaves with alternate arrangement. The leaves are ovate, often puckered, flowers white or pink, and fruits have four small nutlets and based on the features of the plant it was confirmed as *Mentha aquatica*. The identification was made confirmed by plant taxonomist.

Collection of the Leaves From The Plant

After identification of the plant the leaves were separated from the plant. The plant material was dried under dark in shade conditions, without exposing the material to sunlight. After drying, the leaves were powdered using mixer. This leaf powder is passed through sieve no.40 to get fine powder. The powder stored in a cool and dry place until its use.

Preparation of Solvent Extraction

Approximately 40 g of the shade dried powder of plant materials were filled separately in the thimble and extracted with 500 ml ethanol and acetone using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract were weighed and preserved at 4^{o}C in airtight bottles until further use and were used as the test extracts for anti-bacterial assay (Nakano MM *et al*., 1998; Vogt RL *et al*., 2001).

Preparation of Microbial Plate Cultures

Human pathogenic bacteria such as *Pseudomonas aeruginosa* NCIM 5029, *Escherichia coli* NCIM 2563, *Staphylococcus aureus* NCIM 5021, and *Bacillus subtilis* NCIM 2063, were collected from Microbiology Lab, Bhaskar Medical College, Moinabad. All the test bacterial species were maintained on nutrient agar media.

Preparation of Inoculum

The gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C.

Anti-Bacterial Assay

Anti-bacterial activity of ethanol and acetone extracts of *Mentha aquatica* leaf was determined by Agar well-diffusion method. Wells were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 10^6 CFU/ml of bacteria were spread on the solid plates separately with a sterile swab moistened with the bacterial suspension. Then 50 µl of ethanol and acetone extracts of different concentration i.e., 10µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml of *Mentha aquatica* leaf was poured separately in the wells of the inoculated plates. The treatments also included 50 µl of solvents served as control. The plates were incubated for 24 hrs at 37ºC and zone of inhibition if any around the wells was measured in mm (Itah AY *et al*., 2005; Chatterjee I *et al*., 2001).

RESULTS AND DISCUSSION

The anti-bacterial activity against gram negative and gram positive bacteria, of *Mentha aquatica* was studied. The ethanol and acetone extracts of *Mentha aquatica* was very effective against *Staphylococcus aureus* and *Escherichia coli*. The results showed unique characters of the plants in inhibiting bacterial growth.The ethanol and acetone extracts of leaves of *Mentha aquatica* presented a better inhibitory effect on the test organisms. *Escherichia coli* NCIM 2563 and *Staphylococcus aureus* NCIM 5021, were most susceptible when compared to *Bacillus subtilis* NCIM 2063 and *Pseudomonas aeruginosa* NCIM 5029. The active substance causing the inhibitory effect which could have been higher in the leaves. The use of acetone as extracting solvents proved to be more efficient in extracting the active compounds than ethanol. The results of anti-bacterial activity by Agar diffusion method of both the plant extracts against selected bacteria as shown in Table 1 & 2 and graphically represented in graph 1 & 2 respectively.

Table 1. Anti-bacterial activity of ethanol extract of *Mentha aquatica* leaf

Diameter of Zone of inhibition of ethanol extract of *Mentha aquatica* by Agar Well Diffusion Assay					
S. No	**Concentration (µg/ml)**	***E.coli* (mm)**	***B.subtilis* (mm)**	***P.aeruginosa* (mm)**	***S.aureas* (mm)**
1	**10**	2	1	1	2
2	**20**	7	3	2	4

3	**30**	8	4	4	6
4	**40**	9	5	4	7
5	**50**	10	6	5	8

Table 2. Anti-bacterial activity of acetone extract of *Mentha aquatica* leaf

Diameter of Zone of inhibition of acetone extract of *Mentha aquatica* by Agar Well Diffusion Assay					
S.No	**Concentration (µg/ml)**	***E.coli* (mm)**	***B.subtilis* (mm)**	***P.aeruginosa* (mm)**	***S.aureas* (mm)**
1	**10**	**4**	**2**	**5**	**5**
2	**20**	**5**	**4**	**4**	**4**
3	**30**	**7**	**6**	**2**	**3**
4	**40**	**8**	**8**	**2**	**2**
5	**50**	**10**	**10**	**1**	**2**

Figure 1. Anti-bacterial activity of acetone extract of *Mentha aquatica* leaf

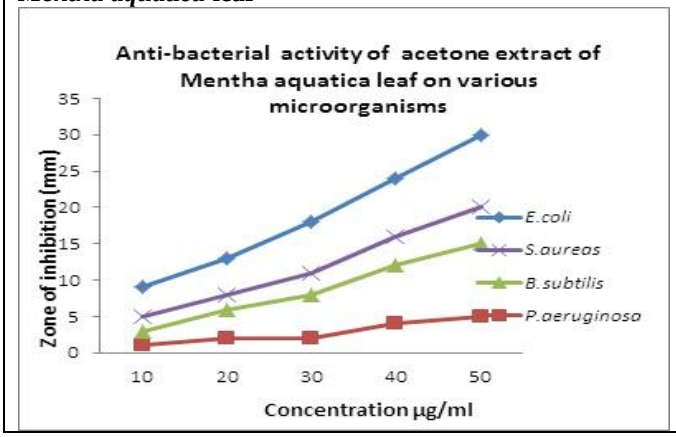
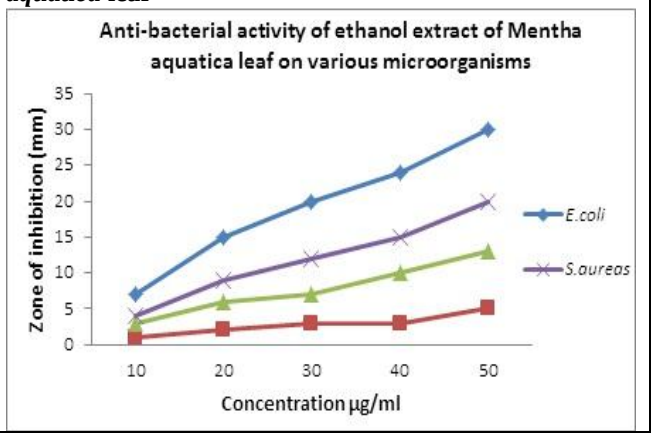


Figure 2. Anti-bacterial activity of ethanol *Mentha aquatica* leaf



CONCLUSION

Plants are important source of potentially useful for the development of new chemotherapeutic agents. The plant extracts showed the highest activity against E.Coli and *Bacillus subtilis* indicating that these plants are good source of anti-bacterial agents for the treatment of certain bacterial diseases.present study implicit the observation that menthe aquatic showed promising anti-bacterial activity against various microorganisms. It was also found that the anti-bacterial activity of extracts increased with increasing concentration. However, further experimental and research work on these plants and their extracts are needed to specify the pharmacological implication.

Extracts obtained from *Mentha aquatica* was observed equally effective against the gram positive and gram negative bacteria. Present investigation reveals that *Mentha aquatica* may be alternative as a preservative in Food Industries since it is equally effective against the gram positive and gram negative bacteria. So it is significant to exploit a novel anti-bacterial drug from these medicinal plants.

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