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# Evaluation of *in vitro* antibacterial activity of *Mentha Longifolia* extracts

Muhammad Ibrar*, Hina Nazir, Kosar Tammana, Jehangir Khan, Palwasha, Aiman Zafar, Ume Roman, Imran Aman, Muhammad Akif, Abid Hussain, Azam Hayat, Mujaddad-ur-rehman

Department of Microbiology, Hazara University Mansehra, Pakistan

***Corresponding author e-mail:** mikhan457@yahoo.com

**ABSTRACT**

The aim of the current study is to investigate the antibacterial activity of *Mentha Longifolia* extract against commonly encountered pathogenic bacteria. The principle focus is on naturally occurring substance and their derivatives especially flavonoids present in plants which are reported to possess good antibacterial activity. The extracts were tested for antibacterial activity against different selected bacteria by disk diffusion method. The results showed that the cold water flower extract exhibited a relatively strong antimicrobial activity against both gram positive and gram negative bacteria tested. The ethanolic extract of *M. Longifolia* also showed good antibacterial activity against both gram positive and gram negative. *E. coli* was the most sensitive bacteria among all and *E. faecalis* was recorded as most resistant bacteria. Present study reveals significantly higher broad-spectrum antibacterial activity of *M. Longifolia* and such findings could be considered a valuable support in the treatment of UTI and respiratory tract infections.

Key words: plant extracts, common pathogenic bacteria, bactericidal activity, *Mentha Longifolia*.

## INTRODUCTION

Antimicrobial resistance by pathogenic bacteria is a major health problem nowadays (Hausner *et al.*, 1999). This increasing drug resistance led the scientists to search new sources of treatment by natural products with greater spectra of biological activities (Abad *et al.*, 2007). The genus *Mentha Longifolia* is a member of the family *Lamiaceae* and subfamily *Nepetoideae* with approximately 14-25species (Gobert *et al.*, 2002; Harley *et al.*, 1972).*Mentha longifolia* is perennial herb extremely variable in height 40-120 cm height. Stem is white or grey-villous, leaves are shortly petiolate or sessile (Harley *et al.*, 1972). The aerial parts of *Mentha L.* adult plants are commonly used as medicine for the treatment of chest inflammations, cold, cough, and asthma. Also used externally to treat swollen gland and wounds (Ikram and Haq, 1980). Mint extracts provide defense against oxidative damages and commonly used as food flavoring additive (Doman *et al*., 2003). *Mentha* spp. have been used for the treatment bronchitis, anorexia, ulcerative colitis, stimulant, carminative, diaphoretic, antiemetic, antispasmodic, analgesic, emmenagogue and anticatharral activities (Gulluce*et al*., 2007). *Mentha* spp. is also used for the treatment of colic, flatulence, menstrual disorders, indigestion, pulmonary infection and congestion, headache, and urinary tract infections. Leaf/stem in boiling water their vapors inhalation relieves nasal or bronchial congestion (Gotshall, R *et al*., 1949). Quercetin3-O-glycoside, Apigenin, Luteolin-7-O-glucoside, Luteolin-7, 3'-O-diglucoside and Kaempfero-3-O-glucoside. These five flavonoids are active against *E. coli, P. aeruginosa, S. aureus*, *B. cereus*, and *B. subtilis* and inhibit their growth (Akroum *et al.*, 2009). The present study was conducted to investigate the antibacterial activity of *Mentha Longifolia* leaf and flower extracts against gram positive *Staph aureus* ATCC6538 and gram negative bacteria *P. auroginosa ATCC74303, K. pneumonia ATCC700603, E. faecalis ATCC35824 and Escherichia coli* ATCC 25922.

## MATERIALS AND METHODS

The current research work was conducted at the Department of Microbiology, Hazara University, Mansehra, Pakistan. The sample plant was collected from Mansehra (Marguzar field). Flower and leaves were used to obtain the extract. The test Micro organisms were kindly provided by Dr. Malik Mujaddad Ur Rehman along with the respective ATCC numbers. Three different solvent were used in the present study to obtain the plant extract i.e. ethanol, hot water and cold water.

**Preparation of Plant Extract:** The leaves and fruits collected from *Mentha Longifolia* were first thoroughly washed with distilled water. Four different extracts of *Mentha Longifolia* were used during the current study i.e. 70% ethanolic leaf extract, leaf hot water extract, leaf cold water extract and flower cold water extract. The samples were air dried at room temperature in shady place and powdered using electric blender. The aqueous extracts were obtained by using 10g of each sample dissolved in 100ml in 70% ethanol and sterile distilled cold and hot water. The extracts were tightly covered with aluminum foil and were allowed to rotate on shaker for 2 days at 150rpm. Afterwards, the extract were filtered from muslin cloth and centrifuged at 4500rpm for 5 minutes. The process of centrifugation was repeated three times. The filtrate was collected and the solvent was removed by rotary vacuum evaporator as described previously (Adebayo and Ishola, 2009).

**Antibiogram Analysis:** Antibiogram analysis was performed to evaluate the antimicrobial properties of plant extract with the help of disc diffusion method. In disk diffusion method, each dilution of bacterial culture was spread by swab on Muller-Hinton agar plate before putting the paper disk on petri dishes. Sterile 6mm filter paper disk were seeded with three dilutions of each extract. At least 20 µl of the extract was used per disk and the disks were allowed to dry before seeding on petri plates. Sterile water discs were employed as negative controls. Subsequently, the petri dishes were incubated at 37°C for 24 hours to check the zones of inhibition. Inhibition zones larger than 15mm were reported as strong antimicrobial activity, 10-15mm as moderate and inhibition zones below 10 mm were reported as weak antimicrobial activity (Bauer *et al*., 1966).

**Statistical Analysis:** Statistical analysis was performed by using Microsoft excel.

## RESULTS

The present study was carried out to check the antibacterial activity of *Mentha Longifolia* leaves and flower extracts by disk diffusion method. The results shows that the flower extract was more active and posses good activity against *K. pneumonia, E.coli, P. auroginosa, S.aureus* and *E. faecalis*, followed by ethanolic extract with activity against Gram positive as well as Gram negative bacteria. Leaf hot water and leaf cold water also showed a considerable antimicrobial activity against both Gram positive and Gram negative.

Gram negative bacteria such as *E. coli* and *P. auroginosa* were found to be the most sensitive bacteria amongst all the different tested bacterial showing 13-25mm and 6-17mm zones of inhibition respectively. While *Klebsiella sp.* and *Enterococcus faecalis* showed sensitivity to flower extract with 19mm and 13mm respectively. Gram positive bacteria like *Staph aureus* also showed good antimicrobial susceptibility to all the extracts with 10-17mm. In contrast, *Klebsiella pneumonia* and *Enterococcus faecalis* were found to be resistant to leaves extract of *Mentha Longifolia*. Furthermore, *P. auroginosa* shows little susceptibility to leaf extract of *Mentha Longifolia.*

## DISCUSSION

Herbal medicines are readily available sources for primary health care system. It is now well established that many species of plants containing substances of medicinal and therapeutic value that have yet to be discovered, though large number of plants are constantly being screened for their antimicrobial activity. Such plants may prove to be a rich source of flavonoids with possible antimicrobial activities, but more pharmacological investigations are necessary in this regard.

The present study showed a significantly higher antimicrobial activity of ethanolic extract of *Mentha Longifolia* leaf that is comparable with the findings reported by other related studies (Cushnie*et al.,* 2003). The highest antibacterial activity of ethanolic extract is possibly due to the high contents of flavonoids present in it. The thin layer chromatography reveals that there is high number of flavonoids and phenolic acids which indicate that *Mentha Longifolia* has great interest in therapeutic and pharmaceutical industries (Martini*et al.,* 2004).
In the presence study, the zones of inhibition of different extract of *Mentha Longifolia* against *E.coli, P. auroginosa, E. faecalis* and *S, aureus* were 17mm,

13mm, 13mm and 14mm. The results obtained during the current study were similar to recent study by Stanisavljević *et al*., (2013) reporting *E.coli* showing *21mm, P. auroginosa 17mm, E. faecalis 17mm, and Staph aureus* showing*17.5mm* zones of inhibition. The small fraction of difference in diameter of zones may be due to the species they used or may be the minimum inhibitory concentration (MIC). A study reported by Pirbalouti (2010) also shows the antibacterial activity of *Mentha Longifolia* ethanolic extract against *E.coli, P. auroginosa, K. pneumonia* and *S, aureus* with 14mm, 12m, 9mm and 10mm zones of inhibition.

In present study the *Mentha longifolia* extract shows good activity against *E. coli* and *Staph aureus.* Similar results have been reported by Hafedh *et al.*, (2010) who reported more accentuated activity of *Mentha Longifolia* against Gram negative bacteria like *E.coli* and Gram positive bacteria such as bacteria *Staph aureus*.

Water and 95% ethanol extracts showed an antibacterial activity against *Bacillus subtilis, Escherichia coli, P. vulgaris, Salmonella typhosa, P. aeruginosa, S. aureus, Shigella dysenteriae, Mycobacterium tuberculosi* (Jawad *et al*., 1988: Ikram and Haq 1980). Furthermore, other studies have also reported the antibacterial activity of *Mentha Longifolia* against *E.coli, P. auroginosa* and *Staph aureus* (Jawad *et al*., 1988; Mimica *et al*., 2003).

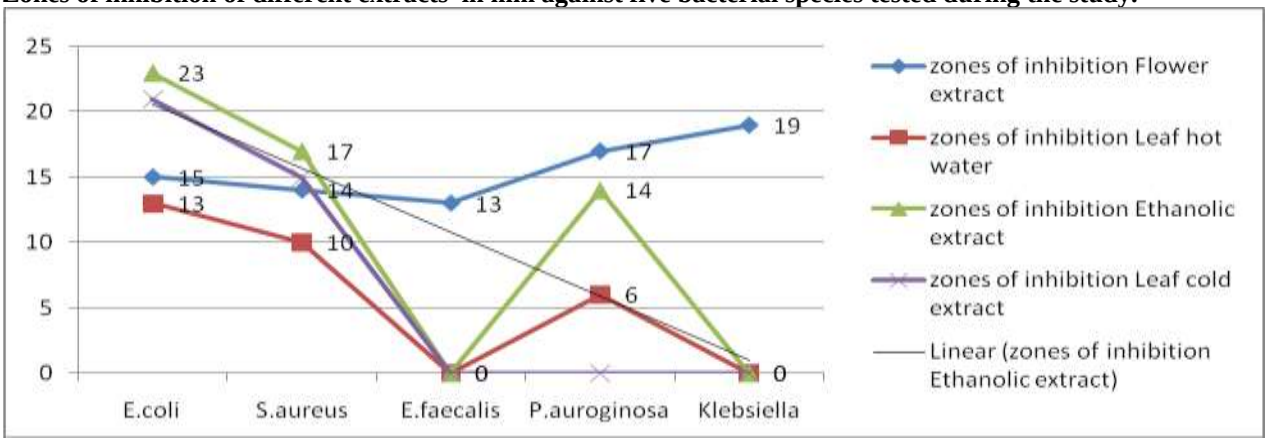
## CONCLUSION

The above study shows the inhibition of some microorganisms which were the causal agent of UTIs, intestinal and respiratory tract infection, indicating that the *M. Longifolia* could be used to cure these infections. It can be recommended for therapeutic purposes and can be used as alternative medicines. So further studies are required to describe the antibacterial activities as in vivo. Also phytochemical, toxicity and proper dose selection studies are required.

**Antibacterial activity of different extracts of *Mentha Longifolia* leaves and flower against different bacteria tested during the current study.**

| Bacteria | 70% Ethanolic leaves extract | Leaf hot water extract | Leaf cold water extract | Flower cold water extract |
|---|---|---|---|---|
| *E.coli* ATCC25922 | 23mm | 13mm | 21mm | 15mm |
| *P. auroginosa ATCC74303* | *14mm* | *6mm* | - | *17mm* |
| *Staph aureus ATCC6538* | *17mm* | *10mm* | *15mm* | *14mm* |
| *Klebsiella pneumonia ATCC700603* | - | - | - | 19mm |
| *Enterococcus faecalis ATCC35824* | - | - | - | 13mm |

**Zones of inhibition of different extracts in mm against five bacterial species tested during the study.**




**1.Flower and Ethanol extract zones of inhibition against *Staph aureus.***

***2.*** **Leaves hot water and leaves cold water against *E.coli.***


**3. Leaf hot and leaf cold water extract activity against *Enterobactor faecalis. 4.*** **Flower extract activity against *Klebsiella pneumonia.***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