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INHIBITORY POTENTIAL OF NINE *MENTHA* SPECIES AGAINST PATHOGENIC BACTERIAL STRAINS

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Abstract

Plants produce secondary metabolites, which are used in their growth and defense against pathogenic agents. These plant based metabolites can be used as natural antibiotics against pathogenic bacteria. Synthetic antibiotics caused different side effects and become resistant to bacteria. Therefore the main objective of the present study was to investigate the inhibitory potential of nine *Mentha* species extracts against pathogenic bacteria. The methanolic leaves extracts of nine *Mentha* species (*Mentha arvensis*, *Mentha longifolia*, *Mentha officinalis*, *Mentha piperita*, *Mentha citrata*, *Mentha pulegium*, *Mentha royleana*, *Mentha spicata* and *Mentha suareolens*) were compared for antimicrobial activities. These *Mentha* species showed strong antibacterial activity against four microorganisms tested. *Mentha arvensis* showed 25 mm and 30 mm zones of inhibition against *Staphylococcus aureus*, *Vibrio cholera* and *Enterobacter aerogens*. Moreover, *Mentha longifolia* showed 24 mm zone of inhibition against *Staphylococcus aureus*. *Mentha officinalis* showed 30 mm zone of inhibition against *Staphylococcus aureus*. 25 mm inhibitory zone was recorded against *Staphylococcus aureus* by *Mentha piperita*. *Mentha royleana* showed 25 mm zone of inhibition against *Vibrio cholera*, while *Mentha spicata* showed 21 mm, 22 mm and 23 mm zones of inhibition against *Staphylococcus aureus*, *Vibrio cholera* and *Enterobacter aerogens*. Moreover most of the *Mentha* species showed zone of inhibition in the range of 10-20 mm.

Key words: *Mentha*, Antibiotics resistance, Anti microbial activities, Pathogenic bacteria.

Introduction

Antibiotics are commonly used for the treatment of serious infections caused by various pathogenic bacteria. According to reports most antibiotics come from microbes and one antibiotic is launched annually (Clark, 1996). In recent years, antibiotic resistance to human pathogenic bacteria has been commonly and widely reported in literature (Davis 1994, Robin *et al*., 1998). To overcome this problem, new antimicrobial compounds with diverse chemical structure and novel mechanism of action are urgently required (Rojas *et al*., 2003).

Man is using plants for the treatment of different ailments since ancient times (Newman, 2000; Shinwari *et al*., 2009; Khalil *et al*., 2014; Ikram *et al*., 2015). Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects (Bibitha *et al*., 2002; Maghrani *et al*., 2005; Shinwari, 2010). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1997; Nimri *et al*., 1999; Saxena & Sharma, 1999; Walter *et al*., 2011). Plants are reported to be the potential source of new antimicrobial agents (Mitscher *et al*., 1987).

Clinical microbiologists, biotechnologist, botanists and biochemists are particularly interested in the field of antimicrobial agents derived from plants; as most of the plant derived photochemicals will find their way to the market and prescribed by physicians as some have already gone through tests in humans (Clark, 1996).

Many studies have been carried out aiming to determine different antimicrobial and photochemical components of medicinal plants and using them for the treatment of various diseases instead of using synthetic drugs to which many microbes have got resistance (Shinwari *et al*., 2009; Fazal *et al*., 2011; Hussain *et al*., 2014; Ahmad *et al*., 2014). The pace of development of new antimicrobial drugs has slowed down in the last ten years while the prevalence of resistance has increased astronomically (Hugo & Russell, 1984).

Today, people all over the world prefer to remain away from chronic stress, pollution and synthetic drugs (Perumalsamy *et al*., 1998). Synthetic drugs on one hand are expensive and difficult to supply and on the other hand microorganisms that are resistant to these drugs are increasing day by day. All these negative aspects of synthetic drugs have turned people's attention towards natural producs and brought alternative and complementary medicines up to date (Dulger *et al*., 1999; Rawat & Uniyal, 2003).

Most of the medicinal plants are yet to be explored for their specific medicinal use (Shinwari & Qaisar, 2011). The medicinal plants can act as an important source for the development of new drugs. Among the estimated 250,000-500,000 plant species, only a small fraction has been investigated pytochemically and even smaller fraction has been subjected to biological and pharmacological screening (Mahesh & Satish, 2008). Random screening of medicinal plants has been very productive as a tool in the area of antibiotic research for searching new biologically active molecules (Fazal *et al.*, 2011, 2012; Ahmad *et al.*, 2014). Investigation on plants used ethnomedicinally is particularly useful in developing

countries where synthetic drugs are expensive and out of reach of poor people. It is obvious that finally these photochemicals will find their way to the market and prescribed by physicians like other antimicrobial drugs (Fazal *et al*., 2011, 2012).

The antimicrobial properties of secondary metabolites from a variety of naturally-grown plants have been assessed (Dorman & Deans, 2000). Studies into the biological activities, mechanism of action and probable uses of plant derived metabolites have regained thrust (Kumar *et al.,* 2008). There appears to be a restoration in the use of conventional approaches to protecting live stock and food from disease, pests and spoilage in developed countries (Shamala *et al.,* 2002). This is particularly true in regard to plant secondary metabolites and their antimicrobial evaluation, as can be seen from the wide range of organisms against which they have been tested (Dorman & Deans, 2000). In the present investigation, methanolic extracts of different *Mentha* species were tested against pathogenic bacteria *Stephylococus aureus, Klebsiella pneumonia, Vibrio cholera and Enterobacter aerogenes*.

Stephylococus aureus leads to different types of hospital acquired infections affecting soft tissues, skin, bloodstream and lower respiratory tract (Plata *et al*., 2009). The treatment of staphylococcal diseases is becoming a worldwide challenge due to the development of resistance to various classes of antibiotics including penicillin followed by the development and spread of strains resistant to the semi synthetic penicillins (nafcillin, methicillin and oxacillin), aminoglycosides, tetracyclines and macrolides (Tenover *et al*., 2001). *Klebsiella pneumonia* causes nosocomial infections of the bloodstream, urinary tract infections, respiratory tract infections and premature infant intensive care unit infections. Multidrug resistant *Klebsiella pneumonia* strains with limited treatment options are becoming a great medical problem globally (Hackstein *et al*., 2013). *Vibrio cholerae* is the main causative agent of cholera (Morris *et al*., 1985). Cholera is an old disease and even today is one of the significant causes of mortality mainly in developing world. Oral or intravenous fluids are the primary treatments of this disease. In severe cases antimicrobial therapy is employed to reduce the volume and duration of diarrhea (Sjolund-Karlsson *et al*., 2011). Like other bacteria, *Vibrio cholerae* is also developing resistance to different classes of antibiotics (Sjolund-Karlsson *et al*., 2011; Shapiro *et al*., 2001; Krishna *et al*., 2006, Shinwari *et al*., 2013), which harbors the cholera therapy. *Enterobacter aerogenes* is the third main cause of respiratory tract nosocomial infections caused by gram-negative bacteria after *Staphylococcus aureus* and *Pseudomonas aeruginosa. Enterobacter aerogenes* strains isolated from hospitalized patients generally exhibit high resistance to broad-spectrum antibiotics (Bornet *et al*., 2000).Therefore novel therapeutic strategies in addition to classical antibiotic therapies are required to combat these pathogens. The current research will help the future researchers and students who are working on *Mentha* species to investigate their potential as antimicrobial agents for various other microorganisms. Moreover, technologies are to be developed to extract these natural antimicrobial compounds from these *Mentha* species. Further research is required to find minimum inhibitory concentrations in different extracts of these species.

Materials and Methods

Plant materials: Nine *Mentha* species of family *Lamiaceae* i.e. *Mentha arvensis*, *Mentha longifolia*, *Mentha officinalis*, *Mentha piperita*, *Mentha citrata*, *Mentha pulegium*, *Mentha royleana*, *Mentha spicata* and *Mentha suareolens* were collected from Herbal Garden, Qarshi Industries (Pvt.) Ltd. Hattar, Swat, Bunir, Khanpur and Peshawar during 2008-2009. These species were identified by the Department of Plant Sciences, Quaid-i-Azam University, Islamabad.

Extraction: Fresh *Mentha* leaves were excised and rinsed with distilled water and dried under shade. Extraction was carried out by simple maceration process. The leaves were taken and grounded in methanol using kitchen blender. This mixture was kept for two weeks at room temperature and then the mixture was filtered twice, using Whatman-41 filter paper. Methanol was completely evaporated by rotary evaporator to obtain the crude extract. Finally, 05, 15 and 30 mg extracts was separately prepared in 10 ml DMSO (Dimethyl sulfoxide) and applied for activity. Standard antibiotics and pure DMSO were used for positive and negative controls.

Bacterial strains used: In current study, one gram-positive strain (*Staphylococcus aureus*) and three gram-negative strains (*Klebsiella pneumoniae*, *Vibrio cholera* and *Enterobacter aerogenes*) were used for antimicrobial activities. These organisms were maintained on nutrient agar medium at 4^{o}C.

Determination of antibacterial activity: Antibacterial activity was determined against four bacterial pathogens by the agar well diffusion method according to the protocols of Fazal *et al*. (2011, 2012). Three different concentrations of methanolic extracts were dissolved in DMSO. Sterile Petri dishes were poured with 20 ml of Mueller Hinton Agar, and after cooling 5 well per plate were made with the help of sterile cork borer (6 mm). Different concentrations (05, 15 and 30 mg) of extracts were poured into each well for antibacterial potential. The plates were incubated at 37°C for 24 hours. The antibacterial activity was determined by measuring the inhibition zone. Standard antibiotics of ciprofloxacin and Azithromycin were used as positive controls.

Results

Many microorganisms, which cause damage to human health, exhibit drug resistance due to inadequate use of antibiotics. Thus, there is a need for the discovery of new substances from natural sources, including plants. When the methanolic leaves extract of *M. arvensis* was tested against *S. aureus*, it showed 20 mm zone of inhibition at 5 mg/10 ml concentration, however, 15 mg/10 ml and 30 mg/10 ml produced 25 mm zone of inhibition against *S. aureus* (Figs. 1-3). The leaves extract of *M. arvensis* showed 15 mm zone against *V. cholera* at 5 mg/10 ml and 15 mg/ 10 ml, while 30 mg/10 ml concentration gave 25 mm zone of inhibition (Figs. 4-6). The leaves extract of *M. arvensis* when tested against *E.*

aerogenes, all the concentrations showed remarkable inhibitory zones. 25 mm zone of inhibition was recorded when 5 mg/10 ml and 15 mg/10 ml concentrations were applied, while 30 mm inhibitory zone was produced by 30 mg/10 ml of extract (Figs. 7-9). *M. arvensis* extract i.e. 5 mg/10 ml, 15 mg/10 ml and 30 mg/10 ml showed 15 mm zones of inhibitions against *K. pneumonia* (Figs. 10-12). The leaves extract of *M. longifolia* gave 14 mm, 20 mm and 24 mm zones of inhibitions at the concentrations of 5 mg/10 ml, 15 mg/10 ml and 30 mg/10 ml respectively against the gram-positive bacteria strain of *S. aureus* (Figs. 1-3). When the leaves extract of *M. longifolia* was tested against *V. cholera*, *E. aerogenes* and *K. pneumoniae* it gave 5 mm, 14 mm and 13 mm zones of inhibitions at 5 mg/10 ml concentration, while at 15 mg/10 ml and 30 mg/10 ml concentrations the inhibitory zones were recorded as 10 mm, 14 mm, 15 mm and 15 mm, 15 mm and 20 mm respectively (Figs. 4-12).

The leaves extract of *M. officinalis* was tested against *S. aureus*, *V. cholera*, *E. aerogenes* and *K. pneumonia* which showed 15 mm, 20 mm and 13 mm inhibitory zone at 5 mg/10 ml concentration, while at 15 mg/10 ml concentrations the extract produced 20 mm, 10 mm, 20 mm and 15 mm zones of inhibitions. Furthermore, 30 mg/10 ml concentration exhibited 30 mm, 10 mm, 20 mm and 17 mm zones of inhibitions respectively (Figs. 1-12). Moreover, the 5 mg/10 ml extract concentrations showed 12 mm, 0 mm, 13 mm and 8 mm inhibitory zones against *S. aureus*, *V. cholera*, *E. aerogenes* and *K. pneumonia* but 15 mg/10 ml produced 13 mm, 0 mm, 15 mm and 10 mm zones of inhibition and 30 mg/10 ml showed 25 mm, 0 mm, 23 mm and 15 mm respectively (Figs. 1-12).

M. citrata extracts at concentrations of 5 mg/10 ml showed 10 mm, 11 mm, 17 mm and 9 mm activities against *V. cholera*, *E. aerogenes*, *S. aureus* and *K. pneumonia* while, 15 mg/10 ml exhibited 19 mm, 15 mm, 17 mm and 13 mm zones and 30 mg/10 ml exhibited 25 mm, 19 mm, 21 mm and 16 mm zones of inhibition against these four bacteria (Figs. 1-12). *M. pulegium* yielded 16 mm, 10 mm, 13 mm and 15 mm inhibitory zones at the concentration of 5 mg/10 ml against *S. aureus*, *V. cholera* , *E. aerogenes* and *K. pneumonia* respectively and 19 mm, 15 mm, 15 mm and 15 mm at 15 mg/10 ml while at 30 mg/10 ml, the zones of inhibition were recorded as 20 mm, 15 mm, 19 mm and 20 mm respectively (Figs. 1-12).

M. royleana also exhibited 15 mm, 25 mm, 19 and 15 mm zones of inhibition at 5 mg/10 ml concentrations, however, 15 mg/10 ml showed 19 mm, 25 mm, 19 mm and 19 mm and 30 mg/10 ml exhibited 20 mm, 25 mm, 20 mm and 19 mm zones against these four bacteria. *M. spicata* extract gave 11 mm, 16 mm and 21 mm inhibitory zones at all three concentrations against gram-positive bacteria i.e., *S. aureus*. (Figs. 1-3). All the three concentrations of *M. spicata* showed 8 mm, 13 mm and 22 mm zones of inhibition against *V. cholera* which is a gram-negative bacterium (Figs. 4-6). Similarly, 5 mg/10 ml extracts yielded 17 mm inhibitory zone while, 15 mg/10 ml and 30 mg/10 ml showed 19 mm and 23 mm zones of inhibition against *E. aerogenes* (Figs. 7-9). *K. pneumonia* was exposed to leaves extract of *M. spicata* and produced 11 mm, 15 mm and 18 mm zones of inhibition at all the three concentrations (Figs. 10-12).

M. suareolens leaves extract at 5 mg/10 ml was found less effective against *S. aureus* which showed 13 mm zone while, 15 mm zone was observed at 15 mg/10 ml and 30 mg/10 ml concentrations (Figs. 1-3). Each concentration of the extract of *M. suareolens* showed 10 mm, 12 mm and 13 mm inhibition zones against *V. cholera* (Figs. 4-6). Lower concentrations of extract showed 15 mm inhibitory zone against *E. aerogenes*. However, at higher concentration 20 mm inhibitory zone was recorded against the same bacteria (Figs. 7-9).

Discussion

Many *Mentha* species have been investigated for phytochemical screening and pharmacological actions in various biological systems and some of which confirmed the traditional uses of these species (Shinwari *et al*., 2011). In the cited literature, different *Mentha* species have been used for anti-infection, antimycobacterial, antimicrobial, antifungal, anti-allergic, anti-inflammatory, bladder stone, chills, cholagogue virucidal, constipation, cyclooxygenase inhibitor, diaphoretic, diarrhea, diuretic, dyspnea, dysentery, dyspepsia, flatulence, gall stone, gastrodynia, haemostatic, insect repellent, jaundice, radio-protective, rheumatism, sedative, stomachache, skin allergies, spasm, stimulant, stomach tonic, throat infections and toothache (Naghibi *et al*., 2005). Furthermore, various parts especially the leaves are commonly used as spice and flavor in different food items. They are commercially exploited for breads, salads, herbal teas, soups, flavor liqueurs, cheese and also added to some important cosmetics (Kofidis *et al*., 2006; Moreno *et al*., 2002; Yadegarinia *et al*., 2006). These species have been traditionally used for the treatment of digestive disorders due to its analgesic, antiemetic spasmodic, anti-inflammatory and carminative properties (Gulluce *et al*., 2007; Moreno *et al*., 2002). The phenolic compounds has been found to be the active components in various parts and the essential oils of *M. arvensis*, *M. piperita*, *M. longifolia* and *M. spicata* are potential agents for scavenging toxic free radicals and minimized the pathogenic action of various microorganisms (Ahmad *et al*., 2012; Hosseinimehr *et al*., 2007; Gulluce *et al*., 2007; Dorman *et al*., 2003; Kaur & Kapoor, 2002; Pandey *et al*., 2003).

In the current study, the antibacterial activities of methanolic extracts of nine *Mentha* species were investigated against pathogenic microorganisms and their potency was compared with each other and antibiotics by measuring the inhibition zones and zone diameter. The results are given in Figs. 1-12. The results showed that most of the *Mentha* species had great potential for antibacterial activities against four bacterial strains tested. Most of the methanol extracts of leaves shows best results against *Staphylococcus aureus.* The diameters of inhibition zones for bacterial strains, which were sensitive to the methanolic leaves extracts of nine *Mentha* species, were in the range of 5–30 mm, at concentration 5, 15 and 30 mg/10ml respectively. The results of this study indicate that the genus *Mentha* can be used as a potential source for antibacterial activity and will help in the isolation of new products/drugs.

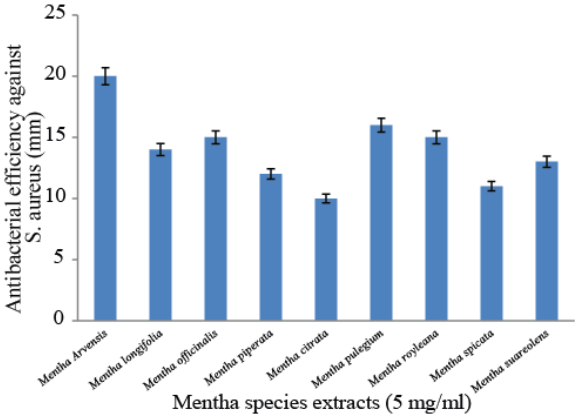

Fig. 1. Antibacterial potential in nine *Mentha* species against human pathogen (*S. aureus*) at concentration of 5mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at p<0.05.

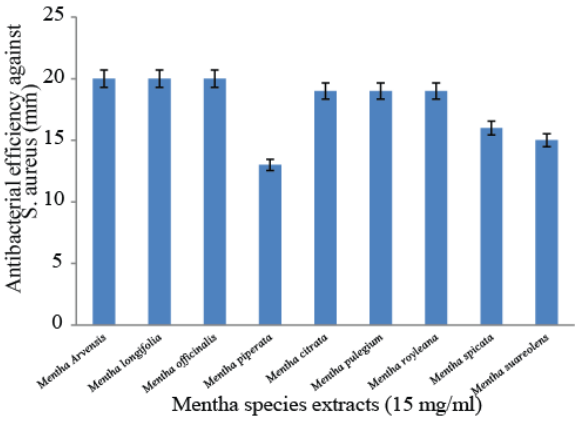

Fig. 2. Antibacterial potential in nine *Mentha* species against human pathogen (*S. aureus*) at concentration of 15mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at p<0.05.

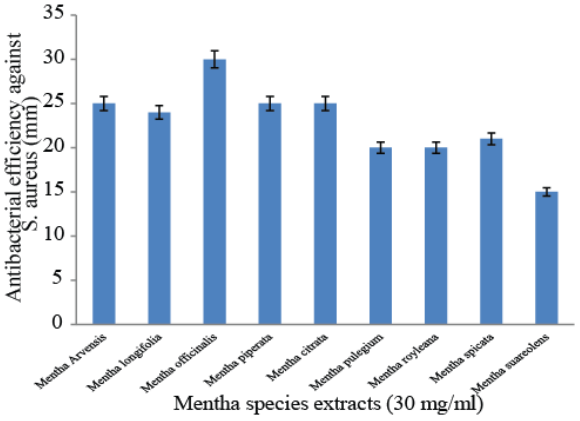

Fig. 3. Antibacterial potential in nine *Mentha* species against human pathogen (*S. aureus*) at concentration of 30mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at p<0.05.

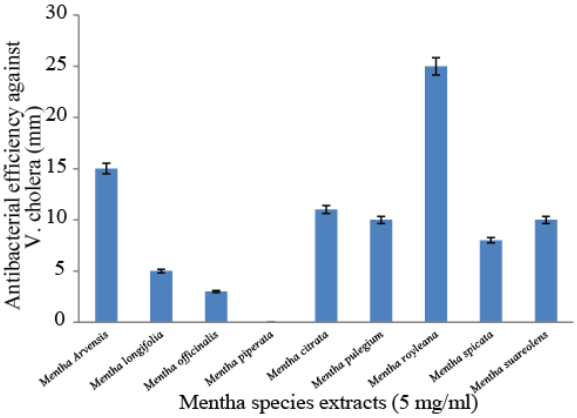

Fig. 4. Antibacterial potential in nine *Mentha* species against human pathogen (*V. cholera*) at concentration of 5mg/10 ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at p<0.05.

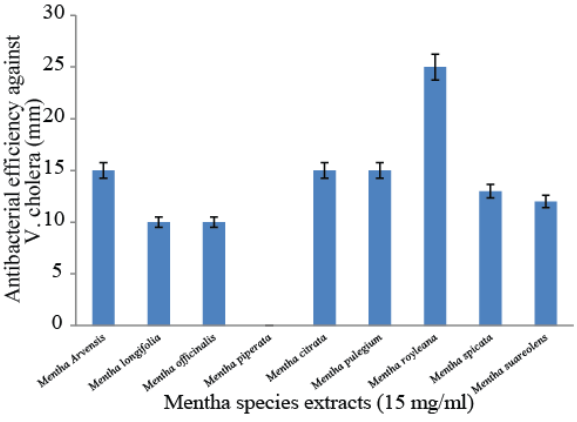

Fig. 5. Antibacterial potential in nine *Mentha* species against human pathogen (*V. cholera*) at concentration of 15mg/10 ml. Mean values with standard errors were taken from triplicate experiments. Each data with common letters are not significantly different at p<0.05.

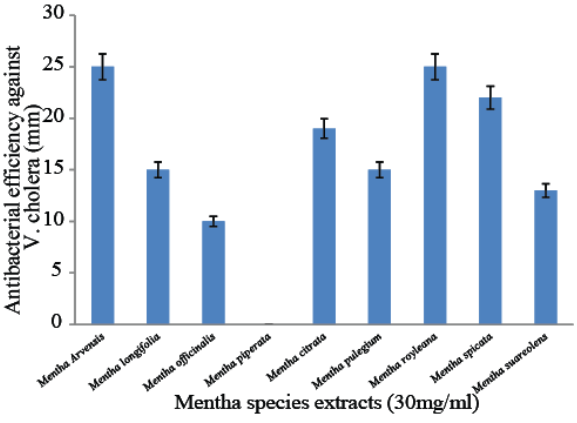

Fig. 6. Antibacterial potential in nine *Mentha* species against human pathogen (*V. cholera*) at concentration of 30mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at p<0.05.

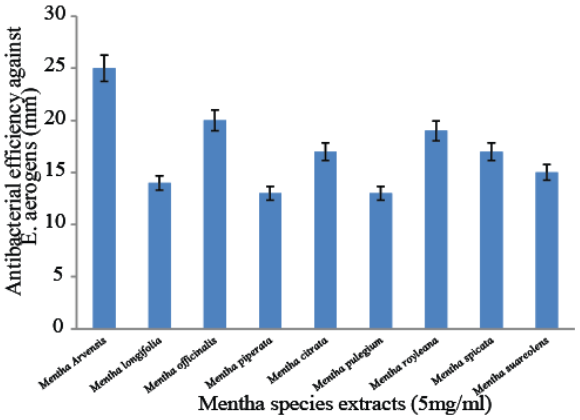

Fig. 7. Antibacterial potential in nine *Mentha* species against human pathogen (*E. aerogens*) at concentration of 5mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at $p<0.05$.

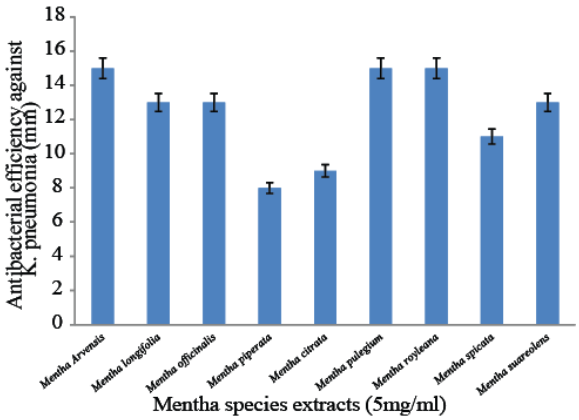

Fig. 10. Antibacterial potential in nine *Mentha* species against human pathogen (*K. pneumonia*) at concentration of 5mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at $p<0.05$.

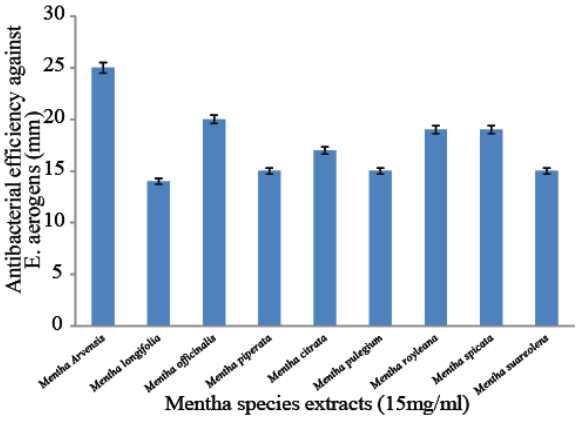

Fig. 8. Antibacterial potential in nine *Mentha* species against human pathogen (*E. aerogens*) at concentration of 15mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at $p<0.05$.

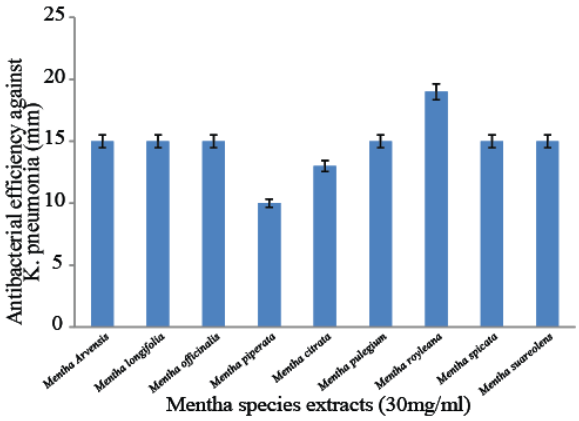

Fig. 11. Antibacterial potential in nine *Mentha* species against human pathogen (*K. pneumonia*) at concentration of 15mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at $p<0.05$.

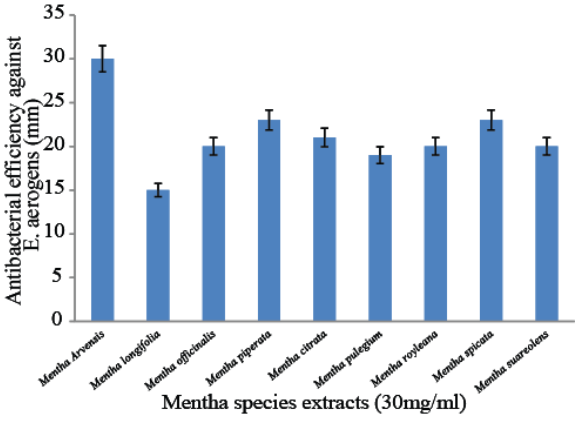

Fig. 9. Antibacterial potential in nine *Mentha* species against human pathogen (*E. aerogens*) at concentration of 30mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at $p<0.05$.

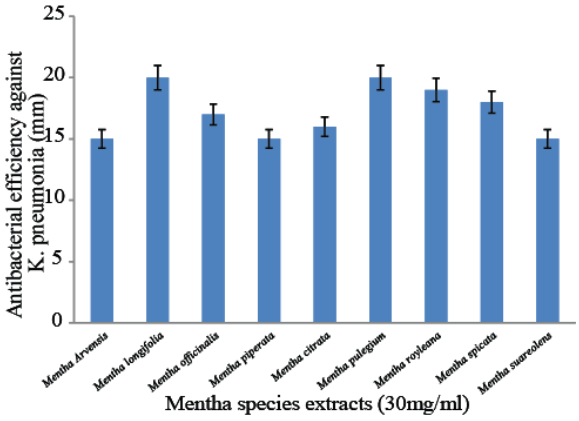

Fig. 12. Antibacterial potential in nine *Mentha* species against human pathogen (*K. pneumonia*) at concentration of 30mg/10ml. Mean values with standard errors, were taken from triplicate experiments. Each data with common letters are not significantly different at $p<0.05$.

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