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SCREENING OF ANTIBACTERIAL ACTIVITY OF *Mentha piperita* L.

A. MAHALAKSHMI PRIYA, M. MANIKANDAN, G. KALAISELVI, P. ARUN, P. CHINNASWAMY** AND K. SELVAM*

*Department of Biotechnology, Dr. N.G.P Arts & Science College, Coimbatore, T.N., India
**Institute of Laboratory Medicine, KMCRET, Coimbatre, T.N., India

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Abstract - The antibacterial effect of *Mentha piperita* extract was tested against various bacteria viz, *Escherichia.coli, Klebsiella pneumoniae, Proteus vulgaris,Psuedomonas aeruginosa,Salmonella typhi* and *Staphylococcus aureus* using agar diffusion method. The raw extract, ethanol, ethyl acetate and chloroform extracts of the leaves of peppermint were tested against these organisms for its antimicrobial activity. Among the four types of extracts, ethyl acetate extract showed the highest antibacterial activity. It was active against four organisms namely *E.coli, K..pneumoniae, P.aeruginosa* and *S.aureus*. The chloroform extract produced the highest zone of clearance against *P.vulgaris* with a diameter of about 1.8 mm. The ethanol extract produced zone of clearance against *E.coli* as well as *S. aureus* while the raw extract produced a zone of clearance only against *S. aureus*.

INTRODUCTION

Microorganisms are omnipresent. They are of utmost importance as they have wide applications in the field of medicine, agriculture, industry etc. But the ill effects caused due to various microorganisms are also ubiquitous. The family Enterobacteriacea contains Gram negative peritrichously flagellated or non-motile, facultatively anaerobic straight rods with simple nutritional requirements. There are a variety of *E.coli* bacteria present in nature. They are usually found in the intestines of healthy humans and animals. Even though these bacteria offer beneficial properties, there are those variations or strains, that are pathogenic. Infertility, miscarriage and born dead puppies is caused by bacterial factors like *E.coli* in polar foxes (Kopczewski *et al.* 1987).

K. pneumoniae is the most clinically important species and infections are common in hospitals where they cause pneumoniae and urinary tract infections in catheterized patients. Apart from this *K.pneumoniae* is more common in the dental plaque of vegetarians (Sedgley *et al.* 1996), in AIDS patients (Zambom *et al.*1990).

Salmonella can be found in the intestines of farm animals such as cattle and chicken. The prevalence of *Salmonella enteritides* and other *salmonella sp* among Canadian registered commercial chicken broiler flocks (Poppe *et al.* 1991) has been reported. Proteus species (Kopczewski *et al.* 1988 and Birgere *et al.* 1996) has isolated from genital tract swabs, collected in the case of infertility, miscarriage, born dead or weak sucklings from carnivorous female animals.

Pseudomonas species have widely been reputed in their ability to degrade a wide range of aromatic compounds like phenyl, ethyl benzene, toluene, and benzene (Haiglert *et al.* 1992). *S.aureus* is associated with haemolysis, where there is a clear zone around the colony in blood agar plate. *S.intermedius* is also associated in infertility, miscarriage in carnivorous female animals (Kopczewski *et al.* 1988). *Mentha piperita* is one of the world's oldest medicinal herbs and is used in both Eastern and Western traditions. Peppermint leaf and oil are used for folk medicine, as flavouring agents in cosmetics and in pharmaceutical products throughout the world. Peppermint oil is most extensively used of all the volatile oils.

Herbilists consider peppermint as astringent, antiseptic, antiprurtic, antispasmodic, antiemetic, carminative, antimicrobial, and stimulant for respiratory congestion. Peppermint tea is used to treat coughs, bronchitis and inflammation of the oral mucosa and throat. Menthol is the primary component of the essential oil of peppermint. Other constituents include menthone, methyl acetate, menthofuran and limonene. Peppermint herb also

Corresponding adderess - Dr.K.SELVAM*, Head, Dept.of Biotechnology, Dr.N.G.P Arts & Science College, Coimbatore 641 035.

contains flavonoids, phytol, tocopherols, carotenoids, betaine, choline, azulenes, rosmarinic acid and tannins (Leung and Foster, 1996). The present study was done to check the antimicrobial activity of peppermint by extraction depending on various solvents.

MATERIALS AND METHODS

Biochemical tests pertaining to different bacteria were done. The organisms used in this study were *E.coli, K.pneumoniae, P.vulgaris, P.aeruginosa, S.typhi* and *S.aureus* respectively.

The strains were collected from Kovai Medical Center and Hospital (KMCH) and cultures were maintained in nutrient broth for further analysis. The sample, peppermint leaves were collected from Kalappatti village. The leaves were washed and air-dried under shade.

The grams staining biochemical tests were performed. Fermentation of simple carbohydrates, Catalase test, Gelatinase test and IMViC test (indole test, methyl red test, Voges-proskauer test and Simmon's citrate agar test). The extraction of peppermint was done with different solvents and extracts were raw extract, ethanol extract, ethylacetate extract and chloroform extract.

Raw extract was taken by grinding the leaves with distilled water. Ethanol extract were obtained by grinding the leaves with absolute alcohol (100%). The extract of ethyl acetate was taken by grinding the leaves and immersed in ethyl acetate and kept in shaker for overnight. The chloroform extract was obtained by grinding the fresh leaves and immersed in chloroform, which was kept in shaker for over night.

All the extracts were analyzed for their

Table1. Acid / Gas Production

S.No.	Organisms	Glucose	Fructose	Lactose	Sucrose	Maltose
1.	Control	-	-	-	-	-
2.	*Escherichia coli*	A/G	A/G	A/G	A/G	A/G
3.	*Klebsiella pneumoniae*	A/G	A/G	A/G	A/G	A/G
4.	*Proteus vulgaris*	A	A	-	A	A
5.	*Pseudomonas aeruginosa*	A/G	A	-	-	- 6.
	Salmonella typhi	A/G	A/G	A/G	A/G	A/G
7.	*Staphylococcus aureus*	A	A	-	A	-

A/G= acid and gas production, A= acid production

Table 2. Biochemical analysis of clinical isolates

S.No.	Organisms	Catalase Test	Gelatinase Test	Indole Test	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test
1.	Control	-	-	-	-	-	-
2.	*Escherichia coli*	+	-	+	+	+	-
3.	*Klebsiella pneumoniae*	+	-	-	-	+	+
4.	*Proteus vulgaris*	+	-	-	-	-	+
5.	*Pseudomonas aeruginosa*	+	+	-	+	+	+
6.	*Salmonella typhi*	-	-	-	+	-	+
7.	*Staphylococcus aureus*	+	+	-	-	+	+

Table 3. Zone of clearance of crude extract of *Mentha piperita* L

S.No.	Organisms	Diameter of the zone Z1 (mm)	Diameter of the well Z(mm)	Zone of Clearance (Z1-Z) (mm)
1.	*Escherichia coli*	-	0.6	-
2.	*Klebsiella pneumoniae*	-	0.6	-
3.	*Proteus vulgaris*	-	0.6	-
4.	*Pseudomonas aeruginosa*	-	0.6	-
5.	*Salmonella typhi*	-	0.6	-
6.	*Staphylococcus aureus*	1.2	0.6	0.6

Table 4. Zone of clearance of ethanol extract *Mentha piperita* L.

S.No.	Organisms	Diameter of the zone Z1 (mm)	Diameter of the well Z(mm)	Zone of Clearance (Z1-Z) (mm)
1.	*Eschericha coli*	0.8	0.6	0.2
2.	*Klebsiella pneumoniae*	-	0.6	-
3.	*Proteus vulgaris*	-	0.6	-
4.	*Pseudomonas aeruginosa*	-	0.6	-
5.	*Salmonella typhi*	-	0.6	-
6.	*Staphylococcus aureus*	1.1	0.6	0.5

Table 5. Zone of clearance of ethyl acetate extract of *Mentha piperita*L

S.No.	Organisms	Diameter of the zone Z1 (mm)	Diameter of the well Z(mm)	Zone of Clearance (Z1-Z) (mm)
1.	*Escherichia coli*	1.6	0.6	1.0
2.	*Klebsiella pneumoniae*	1.2	0.6	0.6
3.	*Proteus vulgaris*	-	0.6	-
4.	*Pseudomonas aeruginosa*	1.5	0.6	0.9
5.	*Salmonella typhi*	-	0.6	-
6.	*Staphylococcus aureus*	1.0	0.6	0.4

Table 6. Zone of clearance of chloroform extract of *Mentha piperita* L

S.No.	Organisms	Diameter of the zone Z1 (mm)	Diameter of the well Z(mm)	Zone of Clearance (Z1-Z) (mm)
1.	Escherichia coli	-	0.6	-
2.	Klebsiella pneumoniae	-	0.6	-
3.	Proteus vulgaris	2.4	0.6	1.8
4.	Pseudomonas aeruginosa	1.3	0.6	0.7
5.	Salmonella typhi	-	0.6	-
6.	Staphylococcus aureus	-	0.6	-

antimicrobial activity and compared with the control, distilled water. The testing of the bacterial cultures for the antibacterial activity of *M.piperita* was done using agar diffusion method. (Bauer and Kirby, 1966). Agar diffusion method was done by using the nutrient agar medium, which was poured into plates. A well with a diameter of 0.6mm was made by well puncture and the plate was swabbed by the culture. A concentration of 200µL of the four leaf extracts were poured on to the well and kept at 37^0C overnight. Distilled water was kept as control.

RESULTS AND DISCUSSION

Gram staining results indicated that the organisms *E.coli, P.vulgaris, S.typhi, K. pneumoniae* and *P.aeruginosa* were gram-negative rods. The organism *S.aureus* was gram-positive cocci.

The biochemical tests indicated the following results. The organisms *E.coli, K. pneumoniae* and *S. typhi* produced both acid and gas in all the five carbohydrate medium containing glucose, fructose, lactose, sucrose and maltose. The organisms *P.aeruginosa* produced acid and gas in the medium containing glucose, acid in fructose medium and neither gas nor acid in the medium containing lactose, maltose and sucrose. The organism *P.vulgaris* produced acid in all the four medium containing glucose, fructose, sucrose, maltose as the carbohydrate source while produced neither gas nor acid in the medium containing lactose. The organism *S.aureus* produced acid in the medium containing glucose, fructose and sucrose as the carbohydrate source while produced neither gas nor acid in the medium containing sucrose and maltose as the carbohydrate source. The production of acid was indicated by the change in colour of the medium from red to yellow. This is because the indicator phenolphthalein turns from red (alkaline pH) to yellow (acidic pH). The production of gas was indicated by the presence of air bubble in the Durham's tube. The colour of control remained red

(alkaline pH) (Table 1).

All the five organisms *E.coli, K.pneumoniae, S.aureus, P.vulgaris* and *P. aeruginosa* produced effervescence due to the evolution of oxygen due to the breakdown of hydrogen peroxide by the enzyme catalase while *S.typhi* gave a negative result. (Table 2).

The organisms *S.aureus and P. aeruginosa* produced a clear zone around the colony when inoculated in gelatin containing medium due to the hydrolysis of gelatin by the secretion of hydrolytic extracellular enzyme gelatinase that which was absent in the plates containing *E.coli, S.typhi, P. vulgaris* and *K. preumoniae.*

The organism *E. coli* showed a positive result for indole production test due to the production of indole by utilizing the amino acid tryptone while the rest of the used organisms were negative.The organisms *E.coli, S.typhi, P.aeruginosa* showed positive result for methyl red test due to the production of acid while *S.aureus, K. pneumoniae* and *P. vulgaris* showed negative result.

The organisms *E.coli, P.aeruginosa, K.pneumoniae* and *S. aureus* were positive for Voges Proskauer test due to the production of acetylmethylcarbinol while the rest of the used organisms were negative.The organisms *P aeruginosa, P. vulgaris, S.typhi, S. aureus* and *K.pneumoniae* gave positive result for Citrate utilization test indicating their ability to utilize citrate while the other organisms were negative.

The antimicrobial activity of peppermint was observed and noted (Table 3 - 6).

Out of the four types of extracts used, the crude extract of peppermint showed a zone of clearance only for *S.aureus* which measured about 0.6 mm in diameter while produced no zone of clearance in other organisms this is in coincidence with the report showing the antimicrobial activity of *Bridelia ferruginea* against *S. aureus* (Irobi *et al.* 1994).

The ethanol extract of peppermint produced a zone of clearance for *E.coli* and *S. aureus* with a diameter of 0.2 mm and 0.5 mm respectively. The ethyl acetate extract showed the highest activity by producing a zone of clearance against four organisms namely *E.coli, K.pneumoniae, P.aeruginosa* and *S aureus* measuring about 1.0 mm, 0.6 mm, 0.9 mm and 0.4 mm respectively. This is similar to the antimicrobial activity of peppermint. It concerns with volatile oils, catechic tannins, flavonoids and anthraquinones (Diaz *et al.* 1988).

The chloroform extract produced the highest zone of clearance with the highest zone for *P. vulgaris*, It measured about 1.8 mm. The chloroform also produced a zone of clearance for *P. aeruginosa* producing a diamete of about 0.7 mm. This is similar to the antimicrobial activity of *Plicosepalus acaciae* leaves against Gram-negative organisms (Elegami *et al.* 2001).

From the present study it is concluded that *M.piperita* L has got antimicrobial activity against gram positive and gram negative organisms.

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