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Research Article

A COMPARATIVE SCREENING OF ANTIBACTERIAL ACTIVITY OF *ANISOMELES INDICA* WITH *MENTHA PIPERITA* AGAINST HUMAN PATHOGENIC MICRO-ORGANISMS

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ABSTRACT
The antibacterial effect of leaves of *Anisomeles indica* and *Mentha piperita* were tested against various pathogenic bacteria-*E.coli, S.aureus, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* using Agar diffusion method. The aqueous, methanolic, ethanolic, Chloroform and ethyl acetate extract of the leaves of *Anisomeles indica* and *Mentha piperita* were tested against these micro-organisms for their microbial activity. Among the five types of extracts the aqueous and ethyl acetate extract of leaves of *Mentha piperita* showed the highest antibacterial activity (1.8mm). The antibacterial activity of *Mentha piperita* is better than *Anisomeles indica*.

Key Words: *Antibacterial Activity, Anisomeles Indica and Mentha Piperita*

INTRODUCTION
In the past few years a number of investigations have been conducted world-wide to prove antimicrobial activities from medicinal plants (Nascimento *et al.,* 1990). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade with more intensive studies for natural therapies. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds, which are a part of the essential oils (Jansen *et al.,* 1987) as well as tannin (Saxena *et al.,* 1994). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.,* 2003). The infectious diseases mainly caused due to microbial contamination of foods are becoming a major problem in the world, particularly in the developing societies (Burt, 2004; Sokovic and Van Griensven, 2006). The microbial growth in foods not only leads to decrease the nutritive and organoleptic value of food commodities, nevertheless it generates several toxins that are harmful for the health of humans (Celiktas, 2007).
Anisomeles indica (Indian Catmint) is a camphor-scented annual herb. Infusion is useful in affections of the stomach and bowels, in catarrh and intermittent fever and that the juice of the leaves is administrated to children for colic, dyspepsia and fever caused by teething. Inhaling the vapor of the hot infusion induces copious perspiration. A decoction of the plant is an excellent fomentation and used externally as an embrocation in rheumatism arthritis. The fumes of leaves have the property of mosquito repellent.
Mentha piperita, (Peppermint) is herbaceous rhizomatous perennial plant. Peppermint has high menthol content and is often used as tea and for flavoring ice cream, confectionery, chewing gum, and toothpaste. The oil also contains menthone and menthyl esters, particularly menthyl acetate. It is the oldest and most popular flavour of mint-flavored confectionery. Peppermint can also be found in some shampoos and soaps, which give the hair a minty scent and produce a cooling sensation on the skin.
Staphylococcus aureus is a facultative anaerobic. It is frequently part of the skin flora found in the nose and on skin. About 20% of the human population is long-term carriers of S. aureus. S. aureus can cause a range of illnesses from minor skin infections, such as pimples impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as

pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia and sepsis.

Proteus vulgaris is a rod-shaped, Gram-negative bacterium that inhabits the intestinal tracts of humans and animals. It can be found in soil, water and faecal matter.. It is known to cause urinary tract infections and wound infections.

Escherichia coli is Gram negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms).

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin and intestines. *Klebsiella* ranks second to E. coli for urinary tract infections in older persons. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy and rhinoscleroma.

Pseudomonas aeruginosa is a common bacterium, which can cause disease in animals, including humans. It is found in soil, water, skin flora and most man-made environment through-out the world. The symptoms of such infections are generalized inflammation and sepsis. It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oil spills.

MATERIALS AND METHODS

In vitro antimicrobial activity was examined for various extracts of above five medicinal plants. The bacterial strains cultures for the work were collected from the IMTech, Chandigarh in the ampules.

Plant materials were washed separately under running tap water, followed by sterilized distilled water. Then plant material was air dried in the shade and grinded to a fine powder and stored. The extraction of leaves was done with different solvents and extracts were aqueous extract, methanolic extract, ethanolic extract, chloroform extract and ethyl acetate extract.

Aqueous Extract

Twenty-five gram of dried powder of plant materials were macerated separately with 50 ml of sterile distilled water using pestle and mortar. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4ºC until further use.

Methanolic and Ethanolic Extract

Methanolic and ethanolic extracts were obtained by grinding leaves with methanol and ethanol respectively. The macerate was first filtered through four layers of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4ºC until further use.

Chloroform Extract

The chloroform extract was obtained by grinding the fresh leaves and immersed in chloroform, which was kept in shaker for overnight. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4ºC until further use.

Ethyl Acetate Extract

The extract of ethyl acetate was taken by grinding the leaves and roots and immersed in ethyl acetate and kept in shaker for overnight. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min. at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4ºC until further use. Agar-well Diffusion Method tested the antibacterial activity. Briefly 20 ml of Nutrient Agar was poured into the Petri dish and 7 mm well bored in the agar. 100µl of

different concentrations of extracts was poured into the wells. The plates were incubated for 24 hrs. at 37°C and the zone of inhibition was measured in mm.

RESULTS

Aqueous Extract

Microorganisms	**Leaf(*M. piperita*)**			**Leaf*(A.indica*)**		
	A1 (mm)	**A2 (mm)**	**A2−A1**	**A1(mm)**	**A2 (mm)**	**A2−A1**
Escherichia coli	7.0	7.0	0.0	7.0	7.0	0.0
Klebsiella pneumoniae	7.0	7.0	0.0	7.0	7.0	0.0
Proteus vulgaris	7.0	7.0	0.0	7.0	7.3	0.3
Pseudomonas aeruginosa	7.0	7.5	0.5	7.0	7.9	0.9
Staphylococcus aureus	7.0	7.8	**1.8**	7.0	7.0	0.0

Methanolic Extract

Microorganisms	**Leaf(*M. piperita*)**			**Leaf(*A.indica)***		
	A1 (mm)	**A2 (mm)**	**A2−A1**	**A1(mm)**	**A2(mm)**	**A2−A1**
Escherichia coli	7.0	8.3	1.3	7.0	8.2	1.2
Klebsiella pneumoniae	7.0	8.3	1.3	7.0	7.0	0.0
Proteus vulgaris	7.0	7.0	0.0	7.0	7.5	0.5
Pseudomonas aeruginosa	7.0	7.0	0.0	7.0	8.0	1.0
Staphylococcus aureus	7.0	7.5	0.5	7.0	7.0	0.0

Ethanolic Extract

Microorganisms	**Leaf(*M. piperita*)**			**Leaf*(A.indica*)**		
	A1 (mm)	**A2 (mm)**	**A2−A1**	**A1 (mm)**	**A2(mm)**	**A2−A1**
Escherichia coli	7.0	7.2	0.2	7.0	8.0	1.0
Klebsiella pneumoniae	7.0	7.0	0.0	7.0	8.2	1.2
Proteus vulgaris	7.0	7.0	0.0	7.0	8.1	1.1
Pseudomonas aeruginosa	7.0	7.0	0.0	7.0	7.5	0.5
Staphylococcus aureus	7.0	7.5	0.5	7.0	7.0	0.0

Chloroform Extract

Microorganisms	**Leaf(*M. piperita*)**			**Leaf*(A.indica*)**		
	A1(mm)	**A2(mm)**	**A2−A1**	**A1 (mm)**	**A2 (mm)**	**A2−A1**
Escherichia coli	7.0	8.0	1.0	7.0	7.5	0.5
Klebsiella pneumoniae	7.0	8.4	1.4	7.0	7.0	0.0
Proteus vulgaris	7.0	8.8	**1.8**	7.0	7.9	0.9
Pseudomonas aeruginosa	7.0	7.5	0.5	7.0	8.5	**1.5**
Staphylococcus aureus	7.0	7.0	0.0	7.0	7.0	0.0

Ethyl Acetate Extract

Microorganisms	**Leaf(*M. piperita*)**			**Leaf*(A.indica*)**		
	A1 (mm)	**A2 (mm)**	**A2−A1**	**A1 (mm)**	**A2 (mm)**	**A2−A1**
Escherichia coli	7.0	8.8	**1.8**	7.0	7.0	0.0
Klebsiella pneumoniae	7.0	8.2	**1.2**	7.0	7.0	0.0
Proteus vulgaris	7.0	8.0	1.0	7.0	8.0	1.0
Pseudomonas aeruginosa	7.0	7.5	0.5	7.0	8.0	1.0
Staphylococcus aureus	7.0	7.4	0.4	7.0	7.0	0.0

DISCUSSION

Various extracts of leaves of *A.indica* show the zone of inhibition range from 0.3mm to 1.5mm. Chloroform leaf extract of *A. indica* shows the maximum zone of inhibition of 1.5mm against *P.aeruginosa*, while the lowest zone of inhibition against *E.coli* is shown by aqueous leaf extract of *A.indica* (0.5mm). Against *S.aureus* not any extract is effective. Highest zone of inhibition of 1.5mm is shown by chloroform extract of leaves of *A.indica* against *P.aeruginosa.* Against *K.pneumoniae* only ethanolic extracts show zone of inhibition of 1.2 mm, while 0.5mm, 1.0mm and 1.2mm zone of inhibition is shown by chloroform, ethanolic and methanolic extract against *E.coli*. Ethanolic leaf extract of *A.indica* shows the zone of inhibition of 1.0mm and 0.5mm against *Pseudomonas aeruginosa* respectively..Chloroform extract showed highest antibacterial activity against *P.aeruginosa*..

Against *E.coli* various extracts of *Mentha piperita* shows the zone of inhibition (1mm to 1.8mm). 1.8mm is the highest zone of inhibition shown by leaf extract in ethyl acetate against E.coli. All the extracts show the positive results and value of zone of inhibition is also better against *S.aureus*, which is 1.8mm (shown by aqueous leaf extract of *Mentha piperita*).

it indicates that leaf extract of *Mentha piperita* has the antibacterial activity against *Pseudomonas aeruginosa* also. Against *Klebsiella pneumoniae*, the *Mentha piperita* leaves extract is effective only in the presence of methanol, chloroform and ethyl acetate and the values are 1.3mm, 1.4mm and 1.2mm respectively i.e. the chloroform leaf extract of *Mentha piperita* shows the better value against the *K.pneumoniae*. Out of the five types of extracts used, the aqueous extract of peppermint showed a zone of clearance of only against *S.aureus* and *P.aeruginosa*, which measured 1.8mm and 0.5mm in diameter respectively.

From the present study it is concluded that *M. piperita* has better antibacterial activity than *A. indica* against human pathogenic organisms.

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