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Antimicrobial Activity of Mentha Arvensis L (Pudina) Against on Gram Negative Bacteria

KEYWORDS: Strain of E.coli, Pseudomonas aeruginasa, Klebsiella pneumoniae, Agar well diffusion method, Mentha Arvensis L, solvents extract

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ABSTRACT *The present study has been designed with the objective to examine the Antimicrobial activity of Mentha Arvensis L.(family lamiaceae) against the medically important gram negative bacterial strain like E.coli , Pseudomonas aeruginosa, Klebsiella pneumoniae by agar well diffusion method on Nutrient agar media using some solvent extract like Butanol, Chloroform, DMSO.*

Introduction:
Due to chaotic use of antimicrobial drugs rate of resistance in human pathogenic microorganisms has increased dramatically (Monroe and Polk, 2000; Parekh and Chanda, 2007). Indian medicinal plants are regularly used in various system of medicine because of minimal side effect and cost effectiveness which provide scientific support to the therapeutic use of the plants in tribal medicine (Rajlakshmi et al., 2003).

Mentha Arvensis L belongs to the family Lamiaceae, which is major source of antimicrobial compound. It is also called Menthol mint, japanese mint, corn mint an essential oil is bearing crop is cultivated for the natural menthol, which is widely use in pharmaceutical, cosmetics and flavoring industries.(B Rachel, sugandhi, meera bai, 2011) .It is a erect branched perennial herb with running rootstock rigid branching stem up to 75cm tall. The leaves of the plant are common edible aromatic herb and used in indigestion and rheumatic pain. The plant used to treat liver and spleen diseases, Asthma and Jaundice. The oil is 5% by distillation of leaves, which contain 40-50% menthol. The oil is Antiseptic, Carminative, Refrigerant, stimulant diuretic. Menthol is used for stomach disorders and ointment for headache (Ritish NAIR, Sumitra CHANDA 2007).

The use of plant and their extract in the treatment of diseases back to 460- 370BC when Hippocrates practiced the art of healing by use of plant drugs (Sofowora, E.A. 1982,; B.Rachel 2007 . In addition, the plant Is rich in wide variety of secondary metabolites. Such as tannins, phenols, steroids, flavonoids and volatile oils, were found in-vitro to have antimicrobial properties.(Blanc et al, 1972;Baslaset,al 1980,;Yoshida et al).

In India, an use of medicinal plant from ancient time. Today there is renewed interest in traditional medicine and increasing a demand for more drugs from plant sources this is mainly due to a current widespread belief that, Green medicine is safe and more dependable than the costly synthetic drug.

Botanical information of M. Arvensis*:*

Plant species	: –	Mentha Arvensis L.
Family	: -	Lamiaceae
Common name	:-	Pudina
Part use	: -	Leaves

Phytochemical analysis: Tannins, phenols, Steroids, Flavonoids and volatile oil.
The search for compound with antimicrobial activity has gained increasing important in recent times. (Davis, 1987:7:947)There has also been a rising interest in research for natural product from plant for the discovery of antimicrobial and antioxidant agent in last three decades and in recent time. (Dapkevicus et al.1998:77:140-6)

The objective of this research is to evaluate the potential of Mentha Arvensis L plant extract against the bacterial strain.

Material and Methods*:*
Plant Materials:
Fresh leaves of Mentha Arvensis L were collected from the normal field of Nagpur District, Maharashtra. Fresh plant material was washed under the running tap water.

Micro-organisms used:
The strains of gram negative bacteria are *E.coli, Klebsiella pneumoniae, Pseudomonas aeruginasa* obtained from government medical college, Nagpur, India. All the micro-organisms were maintained at 4^0C on nutrient agar slant.

Preparation of bacterial culture:
A loop full of bacterial culture which has been taken from pure slant cultures with the help of an inoculating needle and mixes it with sterile distilled water in test tube under sterilized condition .The content is mixed thoroughly until a suspension is formed.

Extract preparation:
i) Aqueous extract – 10 gm of fresh leaves was extracted in 50 ml of sterile distilled water. Boil it up to making a crud drug, then filtered it and store at 4^0C in air tight bottles.
Ii) Solvent extract – 10 gm of fresh leaves was extracted in 50 ml of each solvent namely Butanol, DMSO and Chloroform. Filtered the extract and store at 4^0C in airtight bottles.

Agar well diffusion assay:
Take loops full of culture was inoculated in nutrient broth test tube and incubate for 24hrs at 37°C. Also prepared the 500 ml nutrient agar medium and autoclave, then it pour up to 25-35 ml in each Petri plates.

Then 1 ml of inoculums (N. broth) was inoculated into the each N. agar plates and spread by using glass rod After15-20 min. excess inoculums was soaking by cotton swab. For agar well diffusion method, a well was made in the seeded plates with the help of a cup-borer. The two well was made in each plate, one for the aqueous or solvent extract and other was only pure solvent. Labeling the plate, the extract was introduced into the well using micropipettes in proper manner. Incubates the plate at 37°C for 48hrThe microbial growth was determined by measuring the diameter of zone of inhibi-

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tion. The control zones were subtracted from the test zones and the resulting zone diameter is shown in the graph. The experiment was done three times and the mean values are presented.

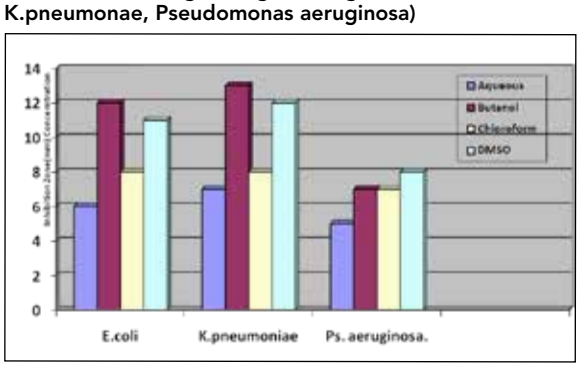
Result: Table 1.
Zone of Inhibition for mentha arvensis L plant against gram negative bacteria by various extract

Plant	Extracts	Microorganisms		
		E.coli	K. Pneumonae	Pseudomonas
M. Arvensis Leaves	Aqueous	6mm	7mm	5mm
	Butonal	12mm	13mm	7mm
	Chloroform	8mm	8mm	7mm
	DMSO	11mm	12mm	8mm

According to the parameters, M. Arvensis L extract was classified as active against *E.coli*, *K.pneumonae*, and partial active against *Pseudomonas aeruginosa*.The Antimicrobial activity of *Pseudomonas aeruginosa* are the lowest and the inhibition zone ranging from 5mm to 10mm. The inhibition zone *of E.coli* and *K.pneumonae* was ranging from 6mm to 15mm.

It can be seen from the observation, the aqueous extract of plant show lowest antimicrobial activity against the all bacterial strains .The zone of inhibition is 4mm to 7mm which is very lowest than the solvent extract.

Graph:
Antimicrobial activity of Mentha Arvensis extract (Aqueous & Solvent) against gram negative bacteria (E.coli, K.pneumonae, Pseudomonas aeruginosa)



Discussion:
The present study had been designed with objective to examine the aqueous and solvent extract of Mentha Arvensis L (Pudina) to show the antimicrobial potential against bacterial strain of *Escherichia coli, Pseudomonas aeruginasa* and *Klebsiella pneumoniae.*

In order to investigate it's in-vitro, the plant extract of Butanol and DMSO show highest antimicrobial activity against the *E. Coli* and *K. Pneumoniae* species and show lowest in the Chloroform. The extract of Butanol and Chloroform show the lowest antimicrobial activity and DMSO shows the highest antimicrobial activity against the *Pseudomonas*.

Also, it can be seen from the graph the Aqueous extract of plant show the lowest antimicrobial potential against all the bacterial species *E.coli, K. pneumoniae* and *Pseudomonas aeruginasa* as compare to remaining solvent extract Butonol, DMSO and Chloroform.

Conclusion:
This study preliminary evaluation of antibacterial activity of M. Arvensis L. It was indicates that M.arvensis L have the potential to generate novel metabolites. The plant extract demonstrated anti bacterial activity could result in the discovery of novel antibacterial agent, and also be used for self medication in domestic settings.

The present findings corroborate that M.arvensis L possess compounds with antimicrobial properties which supports its medicinal use. The results indicate significant capacity and future scope for the use of these plant species against a wide range of microbial populations. The work can be extended to reveal specific secondary metabolites that attributes to their antimicrobial activity. Also further study can be done for determination of toxicity, side effects and pharmaco-kinetic properties of isolated antimicrobial compounds.

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