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Susceptibility of bactrial pathogens to *Mentha citrata* Ehrh

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SUMMARY

The antibacterial activity of methanol, ethanol, Petroleum ether and aqueous extracts from *Mentha citrata* (Lamiaceae) root, stem and leaves were tested by disc diffusion method against *Klebsiella pneumoniae, Bacillus cereus, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus.* Methanolic and ethanolic leaf extracts showed greater activity against pathogenic organisms, than those of acqueous and petroleum ether extract. It exhibited significant antibacterial activity against *E. coli, Staphylococcus aureus and Proteus vulgaris.* Moderate activity was associated with *Bacillus cereus* and *Klebsiella pneumoniae.*

Key words : Antibacterial activity, *Metha citrata*, Leaf extract

Plants provide the principal ingredients used in most of the traditional medicines. Man relied on natural products in general and plants in particular to promote and maintain good health and to fight sickness, pain and disease since time immoreal. With the advances in experimental methods in phytochemical and pharmacology, several medicinal plants were screened for active principles and biological activities (Riley *et al*., 1983). It has been estimated that over half of the world's 25 best selling pharmaceuticals for 1991 owed their origin to a natural source material (O'Neill and Lewis, 1993). Another statistics reveals that approximately 120 plant-derived chemical compounds are currently used as drugs. Many of these are extracted and purified directly from plants (Farnsworth *et al*., 1985). Due to indiscriminate use of antibiotics, the microorganisms have developed resistance to many antibiotics. This leads to serious clinical problems in the treatment of infectious diseases (Davis, 1994). In addition, antibiotics are sometimes associated with side effects and toxicity (Idose *et al.*, 1968; Ahmad *et al.*, 1998). Because of the side effects, drug toxicity and the resistance that pathogenic bacteria built against the antibiotics much recent attention has been paid to extracts and biologically active compounds from medicinal plants. They represent a rich source from which new antibacterial and antifungal chemotherapeutic agents may be obtained. Antimicrobial of plant orgin have enoumous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999; Kokoska *et al.*, 2002).

Mentha citrata Ehrh. Commonly known as bergamont mint, is one of the important aromatic plants of mint family. This taxon cultivation is taken up in Punjab and Utter Pradesh at large scale. According to year 2006, bergamont cultivation in India is done in about 1,200 hectares of land with estimated production of 150 tonnes of bergamont oil. *Mentha citrata*, as it is an hybrid between *Mentha piperata* and *M. viridis* flowering is very rare. Only a few plants in an acre of plot show flowering during the season. The active principle present in this plant is linalool and linalool acetate. They are tritepenoid compounds mainly used in aromatherapy and has many medicinal value. However, there is no report regarding the antibacterial potential of the crude extracts of *Mentha citrata* root, stem and leaf on several bacteria than can cause severe infections and diseases in human and animals.

MATERIALS AND METHODS

Mentha citrata plants were collected from garden of Presidency college, Hebbal, Bangalore, Karnataka, India, which were in turn brought from GKVK, Bangalore. Leaves, stem and roots of these plants were collected, shade dried and crushly powdered

Preparation of crude extracts:

The powdered plant material (10g) were then extracted separately by soaking in, methanol, ethanol petroleum ether and aqueous for 5days. Each mixture was stirred every 12 hrs using sterile glass rod. At the end of fraction, each extract was passed through Whatmann No.1 filterpaper. The filtrate obtained was

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concentrated in vacuum using rotary evaporator.

Pathogenic bacteria tested for antibacterial activity:

– *Klebsiella pneumoniae* (Beijerinck) Taylor: are gram negative, non-motile enterobacteria present in the intestine of human and animals. It causes urinary tract infections, brancho and lobar pneumonia wherein the entire lobe of lung is infected. A marked patchy exudation of a lung parenchyma is noticed in brancho-pneumonia (Robbins *et al*., 1995).

– *Escherichia coli* Castellania and Chalmers: is a gram negative rod, living as a parasite in human and animal intestine (enteropathogenic). It causes enteric diseases, haemorrhage colitis (bloody haemorrhage), urinary tract infections, pyogenic infections and septicemia (Riley *et al*., 1983).

– *Bacillus cereus* Frankland and Frankland: is a gram positive, sporogenous rod shaped bacteria dwelling in water, dust, air and soil. *Bacillus* species other than *Anthrax bacillus* have been implicated in serious infections associated with immunosuppression, parenteral drug abuse and food poisoning. Several noteworthy reviews have been published which describe cases of bacterimia, meningitis, meningo-encephalitis, pheumonia, endocarditis, urinary tract infections, peritonitis, pleuritis and ocular infections of *Bacillus* species (Ihde and Armstrong, 1973; Morris *et al*., 1981; Pearson, 1970; Tuazon *et al*., 1979).

– *Proteus vulgaris* Hauser: is a gram negative, enterobacteria, motile, present in alimentary canal of humans and animals. It invariably causes urinary tract infections.

– *Pseudomonas aeruginosa* (Schroeter) Migula: is a gram negative, aerobic, non-sporic, motile bacillus present in soil, water, sewage, the mammalian gut and plants. It causes nasocomical infections including metabolic, haematological and malignant diseases. Severe epidemic diarrhea of infants, ocular infections, urn infections, cystic fibrisis, hot tub and whirlpool-associated folliculitis and osteomyelitis are caused by *P. aeruginosa.* The patients become susceptible to this organism after prolonged treatment with immunosuppressive agents, corticosteroids, antimetabolites, antibiotics and radiation (Lennette *et al*., 1985).

– *Staphylococcus aureus:* is a gram positive bacteria that causes many skin infections.

Growth media:

The media used for antibacterial test were Nutrient agar, Nutrient Broth Himedia Pvt. Ltd. Mumbai, India. Cultures of bacteria was inoculated into nutrient broth (liquid medium) and incubated at 37^0C for 4hr and suspension was checked to provide approximately 10,00,00 CFU/ml.

Determination of MIC:

Susceptibility of test organism to all the extracts was determined by employing the standard disc diffusion technique (Innette, 1985). The low cost and simple technique of disc diffusion bioassay is advantageous in the determination of the antimicrobial activity of crude drugs (Bauer *et al*., 1966). Whattmann No. 1 filter paper discs of 6mm diameter, placed in dry Petri dish were autoclaved. The sterile filter paper discs were impregnated to each of the test extracts and shaken thoroughly and kept for over night. Later, the saturated filter paper discs were taken out and dried on the laminar air flow bench and carefully placed over the spread cultures and incubated at 37°C for 24h for bacterial growth. Paper discs treated with DMF (negative control) and Streptomycin (positive control) served as control. The zone of inhibition if any, induced by the extracts on growth of microorganisms were calculated and tabulated.

RESULTS AND DISCUSSION

The antibacterial activities of *Mentha citrate Ehrh.* root, stem and leaf extracts against pathogenic bacteria and their potency were assessed by the presence or absence of inhibition zones and zone diameter. The zones of inhibition in diameter (cm) recorded for methanol, ethanol, petroleum ether and aqueous leaf extracts are depicted in Table 1. It is revealed that methanolic and ethanolic extract exhibited significant inhibition against *Proteus vulgaris, Escherichia coli and Staphylococcus aureus,* and less inhibition was associated with *Klebsiella pneumoniae, Bacillus cereus and Pseudomonas aeruginosa.* The aqueous extract of the leaf did not show any antibacterial activity on test organisms. From the results it was obvious that greater activity resides in methanolic leaf extract than root and stem extract. It would generally be expected that a much greater activity against gram positive than gram negative bacteria (Mc Cutchean *et al*., 1992). These differences may be attributed to the fact that the cell wall ink gram positive bacteria consists of single layer whereas gram negative bacteria consists of multilayered structure and quite complex and also act as a barrier to many environmental substances including antibiotics (Tortora *et al.*, 2001). However, in the present study greater activity was observed against gram negative bacteria, while relatively less antibacterial activity was associated with gram positive bacteria. The variation might be due to the difference in the leakage of sodium and

Table 1 : Antibacterial activity of *Mentha citrate* Ehrh

Microorganisms	ME			EE			PE			Aq-E			Strep to mycin
	I	II	III	I	II	III	I	II	III	I	II	III	
Pseudomonas	0.4 ±	o.8 ±	0.9 ±	0.6±	1.0±	0.9 ±	0.5±	0.9 ±	0.5±	0.3 ±	0.3 ±	0.2±	1.6 ±
aeruginosa	0.363	0.537	0.494	0.509	0.396	0.427	0.513	0.447	0.473	0.427	0.413	0.296	0.524
Staphylococcus	0.2 ±	1.2 ±	1.5±	0.7±	0.8±	1.4 ±	0.5±	1.1 ±	1.0 ±	0.3 ±	0.2±	0.5±	1.6 ±
aureus	0.479	0.440	0.484	0.352	0.544	0.479	0.536	0.414	0.563	0.467	0.443	0.502	0.492
E.coli	0.8 ±	0.5±	1.4 ±	0.9±	0.9±	1.2 ±	0.8±	1.2 ±	0.5±	0.5 ±	0.4 ±	0.5±	1.7 ±
	0.484	0.386	0.528	0.372	0.414	0.447	0.414	0.297	0.492	0.488	0.324	0.372	0.324
Proteus vulgaris	0.7±	0.3 ±	1.5±	0.8±	1.1±	1.1 ±	1.1±	1.1 ±	0.8 ±	0.7 ±	0.4±	0.6 ±	1.7±
	0.324	0.455	0.372	0.528	0.528	0.372	0.537	0.296	0.484	0.438	0.517	0.473	0.413
Bacillus subtilis	0.6 ±	0.5±	1.0 ±	0.6 ±	0.8±	1.2 ±	1.1±	1.2 ±	0.5±	0.6 ±	0.6±	0.4 ±	1.7 ±
	0.484	0.386	0.528	0.372	0.414	0.447	0.414	0.297	0.492	0.488	0.324	0.372	0.324

ME= Methane extract, EE= Ethanol extract PE - Petroleum ether extract Aq-E = aqueous Extract I = root II = stem III = leaf
Streptomycin = + ve control for bacteria Data represents an average of 3 experimental sets

potassium ions from the cell membranes of bacteria. This effect may be due to solute uptake, active transport and facilitated diffusion of molecules across the membrane (Oladunmoye *et al.*, 2006). Most commonly the antibacterial compounds are found in plants as secondary metabolites such as alkaloids, flavanoids, terpenoids etc.

In the present study the results were encouraging, as the plant *Mentha citrata* had certain chemical substances that had antimicrobial properties. This results correlates with the observations of previous workers (Mehta *et al.*, 1993; Natarajan *et al.*, 2003; Francis Xavier, 2006).

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