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Comparative analysis of antibacterial and antioxidant activity of *Coriandrum sativum*, *Mentha pipereta* and *Spinacia oleracea*

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The present study was carried out to evaluate the antibacterial and antioxidant activity of the crude ethanolic an acetone extracts of *Mentha pipereta*, *Spinacia oleracea* and *Coriandrum sativum*. The agar well diffusion assay and MIC test were carried out against four strains of bacterial species, *viz., Staphylococcus aureus, E. coli, Bacillus pumulis, and Klebsiella pneumonia* to determine the sample's antibacterial activity. The extracts of the plants at a concentration of 50 µl/disc (200 mg/ml) showed minimum to moderate activity against bacteria indicating a broad spectrum activity. Variable concentrations of ethanolic extract of plant samples were effective against various pathogenic bacteria in MIC test. The result indicated the potential usefulness of these plants especially, in treating bacterial infections and justified the need for further investigations and characterization of the bioactive compounds present in the ethanolic and acetone extracts of the plants. On comparing the three plant materials, the chlorophyll content (both Chl A and Chl B) was found to be maximum in *Spinacia oleracea* leaves. The carotene content was found to be maximum in acetone extract of *Mentha pipereta* leaves, while minimum in *Coriandrum sativum.* Recently, attention has focused on phytochemicals as new sources of natural antioxidants. Therefore, the ethanolic crude extracts of the plant samples were screened for total phenols, flavonoids, and free radical scavenging activity. Free radical scavenging activity was evaluated using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). Significant differences in DPPH scavenging activity were found between the species investigated, ranging from 12.71 per cent to 68 per cent. The highest radical scavenging activity was observed in *Coriandrum sativum* (68% inhibition), followed by *Mentha pipereta* (61.62%) and *Spinacia oleracea* (54.72%). The total phenol content of the investigated species ranged from ±26 to ±75 mg CE/g extract, while flavonoids content ranged from ±22 to ±24 mg CE/g extract. The findings indicated promising antioxidant activity of crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines.

Key words : *Mentha pipereta, Spinacia oleracea, Coriandrum sativum, Staphylococcus aureus, E. coli, Bacillus pumulis, Klebsiella pneumonie,* Flavonoids

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INTRODUCTION

Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity centres with the presence of over 45000 different plant species. Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25 per cent of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80 per cent. An antibacterial is a compound or substance that kills or slows down the growth of bacteria. The term is often used synonymously with the term antibiotic(s); today, however, with increased knowledge of the causative agents of various infectious diseases, antibiotic (s) has come to denote a broader range of antimicrobial compounds, including antifungal and other compounds. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in

a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (McClements and Decker, 2000). Many antioxidant compounds, naturally occurring in plant sources, have been identified as a free radical or active oxygen scavengers (Zheng, and Wang, 2001). A number of plants have been investigated for their biological activities and antioxidant principles (Baris *et al.*, 2006; Saleem *et al.*, 2000). *Spinacia oleracea* is commonly known as spinach which is a flowering plant in the family of Amaranthaceae. Though spinach is most often commonly used as food, it has medicinal value as well. Dietary supplementation with blueberries, spinach, or spirulina reduces ischemic brain damage. Natural antioxidant mixture (NAO), a water-soluble extract obtained from spinach leaves has been shown to have inflammatory, antiproliferative and antioxidative properties in biological systems. *Mentha piperita* commonly known as Pudina, is used for its flavoring and medicinal properties widely throughout various countries of the world. Pudina is currently one of the most economically important aromatic and medicinal crops. It is also known as peppermint, brandy mint, candy mint, lamb mint, balm mint, vilayatipudina or paparaminta. It is strong scented perennial, glabrous herb. The square stems are usually reddish purple and smooth. The leaves are short, oblong-ovate and serrate. It is originally native of Europe, Canada, US and has been naturalised in many parts of India. *Coriandrum sativum* is commonly known as coriander. It has been also called as cilantro or dhania. Coriander, an annual herb, is native to Southern Europe and North Africa to South-western Asia. It is a soft, hairless plant growing to 50 cm tall. The leaves are variable in shape, broadly lobed at the base of the plant, and slender and feathery higher on the flowering stems. The flowers are bornin small umbels, white or very pale pink, asymmetrical, with the petals pointing away from the centre of the umbel longer (5–6 mm) than those pointing towards it (only 1–3 mm long). *Bacillus subtilis*, is a Gram positive, catalase positive bacterium.*K. 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. *Staphylococcus aureus*, it is a facultative anaerobic Gram positive coccal bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passages. It is estimated that 20 per cent of the human population are long-term carriers. *Escherichia coli* is a Gram negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine.

RESEARCH METHODOLOGY

The fresh leaves and petals were collected from the Nursery of School of Forestry and Environment, Sam Higginbottom Institute of Agricutlure, Technology and Sciences, Allahabad which were surface sterilized simply by washing under tap water and Distilled water and dried in shed for 20 days. After drying, leaves and petals were grounded in a grinder mixer to powdered form and stored for further use. The four pre isolated bacterial cultures were collected from Microbial Culture Collection Bank, Sam Higginbottom Institute of Agricutlure, Technology and Sciences. These cultures were sub cultured on NA slants and stored at 4 ^{0}C till use. Plant extracts were prepared using two organic solvents, *viz.* ethanol (70%) and acetone. Minimum inhibitory concentration (MIC) of ethanolic plant extracts against the four pathogenic bacteria was determined (Young and Woodside, 2001). Photosynthetic pigments *i.e.* chlorophyll A, B and total carotenoids in the leaves and petals of medicinal plants were estimated (Saric *et al*., 1976). Free radical scavenging activity was evaluated using L- ascorbic as standard antioxidant using the stable radical DPPH method (Chan *et al.*, 2007). Total phenolic content (Bray and Thorpe, 1954) and total flavonoid content (Morena *et al*., 2000) were determined.

RESEARCH FINDINGS AND ANALYSIS

The salient features of the research as observed have been summarized here under :

The plant samples under study showed antibacterial activity against the tested micro-organisms *viz.*, *Staphylococcus aureus*, *E. coli*, *Bacillus pumulis* and *Klebsiella pneumonia* (Hanen *et al.*, 1998). *Mentha pipereta* showed concentration dependent activity against the tested

Table 1: Comparative analysis of chlorophyll A and B content in the acetone extracts of various plants under study

Plant	O.D. at 662 nm	O.D. at 644 nm	Value of X		Chlorophyll content (mg/g dry weight)	
			Chl A(X_1)	Chl B(X_2)	Chl A	Chl B
Mentha pipereta	1.028	0.451	9.61	4.88	0.480	0.244
Spinacia oleracea	1.940	1.148	17.843	15.573	0.892	0.779
Coriandrum sativum	0.914	0.363	6.216	6.873	0.310	0.343

Chl A = Chlorophyll A, Chl B = Chlorophyll B

microorganisms with the zone of inhibition ranging from 10 to 16 mm at various concentrations but the acetone extract of the sample showed no inhibition against *Bacillus pumulis.* In case of *Spinacia oleracea* the zone of inhibition ranged from 10 to 15 mm but showed no activity against *K. pneumoniae* both for Acetone and Ethanolic Extract. *Coriandrum sativum* zone of inhibition ranged from 10 to 15 mm and inhibition was max against *K. pneumoniae.*

Variable concentrations of ethanolic extract of plant samples were effective against various pathogenic bacteria (Pascal *et al.,* 2001). The least effective concentrations of ethanolic extract of *Mentha pipereta* were found to be 05.55 mg/ml against *S. aureus*, *K. pneumoniae, E. coli* and *B. pumulis.* The least effective concentrations of ethanolic extract of *Spinacia oleracea* leaves were found to be 0.925 mg/ml against *E. coli,* 05.55 mg/ml against *B. pumulis, S. aureus* and *K. pneumoniae.* The least effective concentration of ethanolic extract of *Coriandrum sativum* leaves was found to be 05.55 mg/ml against *S. aureus* and *B. pumulis,* 0.025 mg/ml against *E. coli* and *K. pneumoniae.*

Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light (Uma *et al.,* 2000). In this study, acetone extracts of *Mentha pipereta*, *Spinacia oleracea* and *Coriandrum sativum* leaves were analyzed to determine chlorophyll A and B content (Table 1).

Carotenes contribute to photosynthesis by transmitting the light energy they absorb from chlorophyll and also protect plant tissues by helping to absorb the energy from singlet oxygen, an excited form of the oxygen molecule O_2 which is formed during photosynthesis (Kubo *et al.,* 2004). In this study, acetone extracts of *Mentha pipereta*, *Spinacia oleracea* and *Coriandrum sativum* leaves were analyzed to determine chlorophyll A and B content (Table 2).

Table 2 : Comparative analysis of carotene content in the acetone extracts of various plants under study

Plant	Plant part used	O.D. at 440 nm	Value of x	Carotene content (mg/g dry weight)
Mentha pipereta	Leaves	2.141	6.172	0.309
S. oleracea	Leaves	2.520	2.876	0.144
C. sativum	Leaves	1.164	1.930	0.097

Volume of acetone=10 ml, Weight of sample=200 mg.

The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics, flavonols, and flavenoids and especially DPPH scavenging activity (Aruoma, 1998). DPPH scavenging activity was found maximum at 1.0 mg/ml concentration of ethanolic extract of *Coriandrum sativum i.e.* 68 per cent, which was quiet nearer to the standard used *i.e.* L-ascorbic acid while at the same concentration it was found to be 61.62 per cent and 54.72 per cent for *Mentha pipereta* leaves and *Spinacia oleracea* leaves, respectively (Fig. 1 and Table 3).

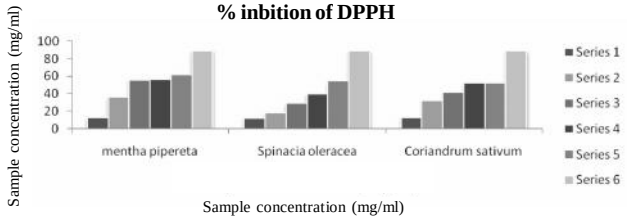

Fig. 1: Comparative analysis of free radical seavenging activity of different plant materials at different concentrations

Table 3: Comparative analysis of free radical scavenging activity of different plant materials at different concentrations

Plant	Material used	Concentration (mg/ml)	O.D. at 517nm	% inhibition of DPPH
L-Ascorbic acid	-	1.0	0.221	88.98
Mentha pipereta	Leaves	0.2	1.747	12.91
		0.4	1.273	36.54
		0.6	0.848	56.05
		0.8	0.864	56.92
		1.0	0.830	61.62
Spinacia oleracea	Leaves	0.2	1.751	12.71
		0.4	1.630	18.74
		0.6	1.421	29.16
		0.8	1.201	40.13
		1.0	0.908	54.72
Coriandrum	Leaves	0.2	1.744	13.06
sativum		0.4	1.353	32.55
		0.6	1.166	41.87
		0.8	0.957	52.30
		1.0	0.642	68.00

It has long been known that phenolic compounds are effective antioxidants. It is reported that phenols are responsible for the variation in the antioxidant activity of the plant (Cai *et al.,* 2004). They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorny *et al.,* 2001; Pitchaon *et al.,* 2007). Highest concentration of total phenol was 75mg CE/g present in the ethanolic extract of *Mentha pipereta* at 1.0 mg/ml concentration whereas lowest in ethanolic extract of *Spinacia oleracea* leaves at the same concentration *i.e.* 26mg CE/g. While in case of ethanolic extract of *Coriandrum sativum* leaves the total phenolic content was found to be 30mg CE/g (Table 4).

Table 4 : Total phenolic content of ethanolic extracts of various plant samples under study

Plant	Plant part used	Concentration (mg/ml)	O.D. at 650 nm	Total phenol (mg CE/g extract)
M. pipereta	Leaves	1	0.936	75.00
S. oleracea	Leaves	1	0.420	26.00
C. sativum	Leaves	1	0.458	30.00

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants (Buhler *et al.*, 2000). These are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties (Miliauskas *et al.*, 2004). Highest concentration of total flavonoids was 24 mg QE/g present in the ethanolic extract of *Spinacia oleracea* leaves at 1.0 mg/ml concentration, whereas lowest *i.e.* 22mg CE/g in ethanolic extract of *Mentha pipereta* at the same concentration. While in case of ethanolic extract of *Coriandrum sativum* leaves, the total flavonoids content was found to be 22 mg CE/g (Table 5).

Table 5 : Total flavonoid content of ethanolic extracts of various plant samples under study

Plant	Plant part used	Concentration of plant extract (mg/ml)	O.D. at 415 nm	Total fslavonoids (mg QE/g extract)
M. pipereta	Leaves	1.0	0.245	22
S. oleracea	Leaves	1.0	0.259	24
C. sativum	Leaves	1.0	0.249	23

LITERATURE CITED

Aruoma, O.I. (1998). Free radicals, oxidative stress and antioxidants in human health and diseases. *J. American Oil Chem.Soc.*, **75**(2): 199-212.

Baris, O., Golloce, M., Sahin, R., Ozer, H., Killic, H. and Ozkan, H. (2006). Biological activities of essential oil and methanol extract of *Achillea bickersteinii.* (Asteraceae). *Turkish J. Biol.*, **30**: 65-73.

Bray, H.G. and Thorpe, W.V. (1954). Biochemical estimation for phenolic compounds. *J. Antimicrobiol. & Chemotherapy*., **1**: 27-52.

Buhler, D.R., Miranda, C.L., Stevens, J.F., Ivanov, V., Mark McCall., BalzFrei. and Deinzer, M.L. (2000) Antioxidant and pro-oxidant actions of prenylated and nonprenylated chalcones and flavanones *in vitro*. *J. Agric. & Food Chem.*, **48**(9): 3876–3884.

Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci*., **74**: 2157-2184.

Chan, E.W.C., Lim, Y.Y. and Chew, Y.L. (2007). Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. *Food Chem.*, **102**(4): 1214-1222.

Hanen, F., Hafedh, H., Najla, T., Emira, N., Mejdi, S., Riadh, K. and Amina, B. (1998) Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and*Mentha pulegium*) used in the Tunisian folkloric medicine. *World J. Microbiol. & Biotechnol.*, **25**: 2227-2238.

Kubo, I., Fujita, K., Kubo, A., Nihei, K. and Ogura, T. (2004). Antibacterial activity of coriander volatile compounds against *Salmonella choleraesuis*. *J. Agric. & Food Chem.*, **52**(11): 3329-3332.

McClements, J. and Decker, E.A. (2000). Lipid oxidation in oil-water emulsions: Impact of molecular environment or chemical reactions in heterogeneous food system. *J. Food Sci.*, **65**(8): 1270-1282.

Miliauskas, G., Venskutonis, P.R. and Beek, T.A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.*, **85**: 231-237.

Morena, E., Mishra, R.P., Arshad, M. and Sami, A. (2000). Antibacterial properties of *Rosa indica* (L.) stem, leaves and flowers. *Electronic J. Biol.*, **12**(15): 4564-4573.

Pascal, J., Delaquis, K., Stanich, B. and Girard, M.G. (2001). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Agric. & Agri-Food Canada, Pacific Agri-Food Res. Centre*., **23**: 34-35.

Pitchaon, M., Suttajit, M. and Pongsawatmani, R. (2007). Assessment of phenolic content and freeradical scavenging capacity of some Thai indigenous plants. *Food Chem.*, **100**: 1409-1418.

Pokorny, J., Yanishlieva, N. and Gordon, M. (2001). Antioxidants in food. Practical applications. *Cambridge Woodhead Publishing Limited*., **72** (5): 145-171.

Saleem, A., Ahotupa, M. and Pihlaja, K. (2000). Total phenolic concentration and antioxidant potential of extracts of medicinal plants of Pakistan. *Zeitschrift fur Nature forschung*., **56**: 973-978.

Saric, M., Kastrori, R., Curie, R., Cupina, T. and Gerie, I. (1976). Chlorophyll determination. Univ. Unoven Sadu Parktikum is fiziologize Bibjoke, Beagard, Hauncna, Anjig. 215. *Curr. Sci.*, **5**: 23-25.

Uma, B., Prabhakar, K., Rajendran, S. and Sarayu, Y.L. (2000). Antimicrobial activity and phytochemical analysis of *Coriander sativum* against infectious diarrhoea. *Ethnobotanical Leaflets*., **5**(4):12-14.

Young, I.S. and Woodside, J.V. (2001). Antioxidants in health and disease. *J. Clin. Pathol.*, **5**: 176–186.

Zheng,W. and Wang, S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. & Food Chem.*, **49**(11): 5165-5170.