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Screening of Antibacterial and Antioxidant Activity of *Anisomeles indica*

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Abstract: ***The antibacterial and antioxidant effect of leaves of Anisomeles indica was tested against various pathogenic bacteria-E.coli, Proteus vulgaris, Klebsiella pneumonia and pseudomonas aeruginosa using Agar diffusion method. The aqueous, methanolic, ethanolic, Chloroform and ethyl acetate extract of the leaves of A.indica were tested against these micro-organisms for their microbial activity as well as antioxidant activity was identified by DPPH Method. This plant is showing antibacterial and antioxidant activity, both. Methanolic extract of Anisomeles indica shows the better scavenging than aqueous extract.***

Keywords: Antibacterial activity, Antioxidant activity, *Anisomeles indica,* Pathogenic bacteria

1. Introduction

During the last decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicines may be available in India, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs. 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). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.

Anisomeles indica (Indian Catmint) is a camphor-scented annual herb. It is erect about 1-2 m in height, with a 4-angled, pubescent stem. The leaves are thin, ovate, 3-12 cm, long-stalked, and pointed at the tip, with round-toothed margins. The flowers are numerous, crowded, and almost stalk less and occur in spike-like racemes 5-25 cm long and 2-3 cm in diameter. The sepal cup is about 6 mm long, hairy and pointed-toothed and the tube, long and bell-shaped. The flowers are purplish and 1-1.2 cm long. The upper lip is oblong-ovate and the lower lip has two middle lobes.

Infusion is useful in affections of the stomach and bowels and 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.

2. Material and Methods

2.1 Bacterial strains

In vitro antimicrobial activity was examined for various extracts of above two medicinal plants. The bacterial strains of *E.coli, K.pneumoniae* and *P.vulgaris* cultures for the work were collected from the IMTech., Chandigarh. Ampule of culture was revived in nutrient broth for use in the antimicrobial test.

2.2 Preparation of Extracts from Collected Material

2.2.1 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.

2.2.2 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.

2.2.3 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.

2.2.4 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.

2.2.5 Antioxidant activity

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Food antioxidants from plant source like vitamin C, vitamin E, carotenes, phenolic acids; phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate harmful disease. Antioxidant compounds like phenolic acids; polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps understand the functional properties of foods.

2.2.6 The DPPH Method

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2, 2,-diphenyl-1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. Antioxidant compounds may be water-soluble, lipid soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity of foods.

3. Results

Aqueous Extract

Table 1: Zone of clearance of aqueous extract of Anisomeles indica

Microorganisms	*Leaf*		
	A1 (mm)	*A2 (mm)*	*A2−A1*
Escherichia coli	7.0	7.0	0.0
Klebsiella pneumoniae	7.0	7.0	0.0
Proteus vulgaris	7.0	7.3	0.3
Pseudomonas aeruginosa	7.0	7.9	0.9
Staphylococcus aureus	7.0	7.0	0.0

Table 2: Zone of clearance of methanolic extract of Anisomeles indica

Microorganisms	*Leaf*		
	A1 (mm)	*A2 (mm)*	*A2−A1*
Escherichia coli	*7.0*	8.2	1.2
Klebsiella pneumoniae	*7.0*	7.0	0.0
Proteus vulgaris	*7.0*	7.5	0.5
Pseudomonas aeruginosa	*7.0*	8.0	1.0
Staphylococcus aureus	*7.0*	7.0	0.0

Table 3: Zone of clearance of ethanolic extract of *Anisomeles indica*

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

Table 4: Zone of clearance of chloroform extract of *Anisomeles indica*

Microorganisms	*Leaf*		
	A1 (mm)	*A2 (mm)*	*A2−A1*
Escherichia coli	*7.0*	*7.5*	*0.5*
Klebsiella pneumoniae	*7.0*	*7.0*	*0.0*
Proteus vulgaris	*7.0*	*7.9*	*0.9*
Pseudomonas aeruginosa	*7.0*	*8.5*	*1.5*
Staphylococcus aureus	*7.0*	*7.0*	*0.0*

Table 5: Zone of clearance of ethyl acetate extract of *Anisomeles indica*

Microorganisms	*Leaf*		
	A1 (mm)	*A2 (mm)*	*A2−A1*
Escherichia coli	7	7	0
Klebsiella pneumoniae	7	7	0
Proteus vulgaris	7	8	1
Pseudomonas aeruginosa	7	8	1
Staphylococcus aureus	7	7	0

Table 5: Effect of extracts of Anisomeles indica on DPPH radical scavenging assay

Sl. No.	Conc. (µg/ml)	Methanolic extract		Aqueous extract		Ascorbic acid	
		Abs	%	Abs	%	Abs	%
1	2	0.34	10.22	0.257	20.2	0.865	8.46
2	4	0.40	12.67	0.387	18.56	0.811	14.1
3	8	0.11	20.45	0.455	15.14	0.558	40.9
4	16	0.16	25.34	0.345	20.35	0.156	83.4
5	32	0.20	10.11	0.231	35.65	0.065	93.1
6	64	0.16	27.90	0.208	31.44	0.053	94.3
7	128	0.11	18.56	0.116	26.60	0.052	94.4
8	256	0.18	25.45	0.178	25.24	0.041	95.6
9	512	0.20	5.2	0.108	20.77	0.039	95.8
10	1000	0.53	10.00	0.312	18.45	0.039	95.8
11	Control	0.33	0.187	0.505	0.4	0.945	0

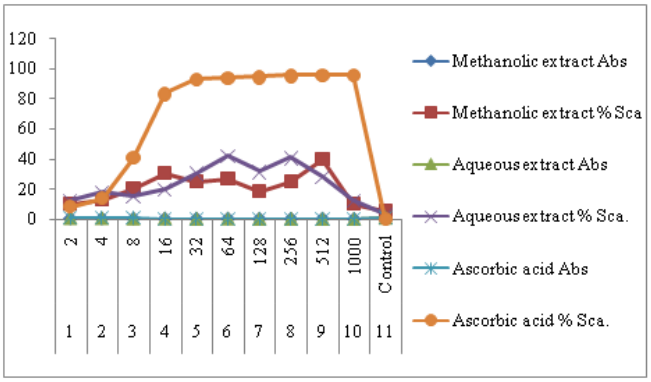

Figure 1: Effect of extracts of DPPH on *Anisomeles indica* radical scavenging assay

4. Antioxidant activity of Anisomeles indica-

The significant concentration of DPPH radical is due to the scavenging ability of methanolic and aqueous extract is shown in tables, which indicates that percentage of scavenging increases as the conc. increases. From the table it is clear that Ascorbic acid has percentage of scavenging from 8.46 to 95.87 when the concentration increases from 2 to 1000µg/ml. Maximum absorbance is given by methanolic extract 0.533 at 1000µg/ml but % scavenging is only 10%. At 4µg/ml. the absorbance is 0.405 and % scavenging is 12%. While methanolic extract shows 18% scavenging at 1000µg/ml, concentration. Fig-1 shows the graphical representation of DPPH radical scavenging assay of aqueous and aqueous extracts. Methanolic extract of *Anisomeles indica* shows the better scavenging than aqueous extract.

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