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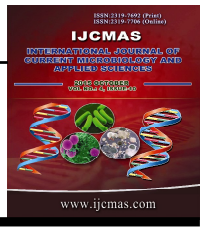

Original Research Article

Study on the Antibacterial, Antioxidant Activities and Phytochemical Analysis of Medicinal Plants in West-Bengal, India

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K e y w o r d s

Medicinal plants, Antibacterial, Antioxidant activity, Phytochemical analysis Ethanol extract

A B S T R A C T

The present study was aimed to evaluate the antibacterial activities of two medicinal plants; *Chenopodium album, Lippia geminata,* against seven human pathogenic bacteria; *Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis, Enterobacter faecalis, Salmonella typhi*. The collected leaves from *Chenopodium album and Lippia geminata* were washed, dried and powdered. Aqueous and ethanol extracts were prepared and observed phytochemical analysis, antibacterial activity and antioxidant activity. Antibacterial action of two plants were tested by Agar well diffusion method and MIC method.

Introduction

Plants have been an essential part of human society since the start of civilization. In the early days of medicine, plants were used for curing many diseases (Huffman, 2001). Plants are known to contain in any of their organ, substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs (Sofowora, 1982). Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times.

Herbal medicine is still the mainstay of about 75–80% of whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and very little side effects. However, the last few years have seen a major increase of their use in the developed world.

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies; this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of therapeutic value and as sources of lead compounds in the drug development. In recent years, multiple drug resistance was developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Davis, 1994; Service, 1995). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions (Ahmad *et al.*, 1998). This situation forced scientists to search for a new antimicrobial substance. Given the alarming incidence of antibiotic resistance in bacteria of medicinal importance (Monroe and Polk, 2001), there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000).

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Clark, 1996). Several screening studies have been carried out in different parts of the world. There are many reports also on the antimicrobial activity of different herbal extracts in different regions of the world. The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants (Parekh and Chanda, 2007). Herbs and spices are known for their antimicrobial and anti oxidative properties. Due to an increasing demand for natural food additives, herbs and spices have emerged as popular ingredients and have a tendency of replacing antimicrobial and antioxidant agent.

Some medicinal plants used in traditional Iran medicine are effective in treating different ailments caused by bacterial and oxidative stress. New antioxidants such as plants phenolic compounds are sought for general health maintenance (Wah Chan *et al.*, 2008). The term phenolics covers a very large and diverse group of chemical compounds. The compounds classified into groups comprise: flavonoids, linins, tannins etc (Vermerris and Nicholson, 2006). Flavonoids are a large group of polyphenolic compounds that occur commonly in plants. In addition; flavonoids based herbal medicines are available in different countries as anti-inflammatory, antispasmodic, antiallergic, antibacterial and antifungal remedies (Rice- Euan and Packer, 2003).

The aim of the present study was to investigate the antibacterial, antioxidant activity and phytochemical analysis of plant extracts of two different plants. These are *Chenopodium album* and *Lippia geminate.*

Materials and Methods

Collection of plant material

The plant materials used for their study were collected from different rural areas of Paschim medinipur, West-Bengal. The taxonomic identities of these plants were confirmed in the Dept. of Botany, Raja N.L. Khan Women's College.

Description of plants

Chenopodium album-Erect, annual herbs, 20-75cm long, lower leaves long petioled,

Flowers are 0.2mm long, fruits depressed globose.

Lippia geminate-Erect aromatic herbs, 2m high, densely puberulent. Leaves are thick-chartaceous. Corolla hypocrateriform, blue or pinkish, 4–5mm long.

Preparation of plant extracts

The plant parts used for extraction preparation are: *Lippia geminate* (whole plant), *Chenopodium album* (whole plant). The fresh plant parts (mentioned above were washed with tap water and then followed by distilled water immediately after collection. The samples were chopped into pieces, dried in shade for few days and then powdered by grinder.

Aqueous extracts preparation: About 60 gram of the dried sample powder of each was mixed with the ratio (w/v) of 1:5 with the solvent water separately. The extraction was carried out in a shaker at 37℃ for 24hrs. The extracts were filtered through Whatmann No.1 filter paper. The extracts were concentrated to dryness by a rotary evaporator at 50℃ and then those are kept at 4℃ for drying.

Ethanol extracts preparation: About 60 gm of dried sample powder of each is mixed in the ratio (w/v) of 1:5 with the solvent ethanol (100%). The extraction was carried out in a shaker at 37℃ for 24 hrs. The extracts were filtered through Whatmann No.1 filter paper. The extracts were concentrated to dryness by a rotary evaporator at 50℃ and then those are kept for freeze drying.

Test microorganisms

The microbial cultures such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Enterobacter faecalis, Bacillus subtilis* and *Shigella dysenteriae* were collected from National Institute of Cholera and Enteric Diseases (NICED). The microorganisms were incubated into Muller Hinton broth for (bioassay and incubated for) 24 hrs at 37℃. The turbidity of the medium indicates the growth of organisms. Then all the cultures were kept at 4℃ until further used.

In vitro antibacterial assay

Determination of MBC: The Agar well diffusion method was used to study the antimicrobial activity of those extracts (Bauer *et al.*, 1966). Lawn culture of *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Enterobactor faecalis, Bacillus subtilis* and *Shigella dysentery* were spread on the Muller Hinton agar using spreader. The wells were cut from the agar plates using a cork borer. The extracts were poured into the well using sterile micropipette. The pure solvents in equal volume served as negative control and streptomycin and chloramphenical solution were poured into another well of each that plates using sterile micropipette. 100% ethanol is also used for positive control where ethanol extracts were used as the solvent extracts. The plates were incubated at 37℃ for 24 hrs. After incubation the diameter of the zone of inhibition were measured by using scale.

Determination of MIC: Four bacterial isolates were selected for MIC studies of each plant because of their potential inhibitory effect against the plant extracts when compared to the other bacterial isolates. The MIC of the plant extracts of *L. geminata* and *C. album*, was determined (Greenwood, 1989). Serial dilution of different concentrations 100, 50, 25 and 12.5 µg/ml were used to determine the MIC. The

MIC was recorded as the least concentrations of the extract that completely inhibited the growth of the test organisms.

About 0.1 ml of the contents of the tubes was further sub-cultured on nutrient agar by pour late method and the contents were incubated for 24 hrs to determine bactericidal or bacteriostatic activity. Bactericidal effect was determined when no growth occurred on the sub-culture medium after the MIC determination.

Phytochemical analysis

The freshly prepared leaf extracts were subjected to preliminary analysis for the presence of phytoconstituents as described (Harborne, 1998). The tests done are as follows –

Tests for alkaloids

Wagner's reagent test- With alkaloids it shows reddish brown precipitated. It is prepared by dissolving 1.27gm of iodine and 2gm of Potassium Iodine in 5ml of water and the final volume is made up to 200ml.

Tests for flavonoids

Test with sulphuric acid- When a drop of H_2SO_4 added to the above, the yellow colour disappears for the presence of flavonoids.

Tests for proteins and amino acids

Biuret test- 2ml of the extracts, 2ml of 10%NaOH solution and 2-3 drops of 1% $CuSO_4$ solution were mixed. Violet or purple colour confirms the presence of proteins.

Tests for tannins and phenolic compounds

Test with ferric chloride- Generally phenols were precipitated with 5% W/V solution of ferric chloride in 90% alcohol and thus phenols can be detected.

Test with gelatin solution- To a solution of tannins (0.5–1%) aqueous solution of gelatin (1%) and sodium chloride (10%) were added. A white buff precipitation appears in the presence of tannins.

Tests for carbohydrates

Benedict's Test- In this method of test for monosaccharide, 5ml of Benedict's reagent and 3ml of test solution when boiled on a water bath, brick red precipitation appears at bottom of the test tube in the presence monosaccharide.

Fehling's test- In this method 2 ml Fehling "A", 2 ml of Fehling "B" and 2 ml of extract were boiled. The presence of reducing sugar confirmed if yellow or brick red precipitate appears at the bottom of the test tube confirms the presence of the monosaccharide.

Tests for steroids and sterols

Libermann Burchard reagent test- In this method of detection, about 2 ml of the solution of extract in chloroform was placed in a dry test tube. Then 2 ml of acetic anhydride and 2–3 drops of conc. H_2SO_4 was added to it and allowed to stand for few minutes. An emerald green colour develops if steroid or sterols and present.

Antioxidant activity

DPPH radical scavenging activity- The free radical scavenging activity of the ethanol extract was measured in terms of radical scavenging ability using the stable 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical method. A solution of 0.1mM DPPH in ethanol was prepared and 1.0ml of this

solution was mixed with 3.0 ml of extract in ethanol containing 10, 20, 30, 40 and 50µg/ml of the extract. The same procedure was followed by using Quercetin as standard. The reaction mixture was vortexed thoroughly. After thirty minutes incubation, the absorbance of the mixture was measured spectrophotometrically at 517nm.

It was compared to control containing same amount of ethanol and DPPH without an extract or standard. The capability to scavenging the DPPH radical was calculated using the following formula (Nmorsi *et al.,* 2007):

% Inhibition = $\{[Ac - Aa]/Ac\}$ X 100

Where Ac is the absorption of the control and Aa is absorption of the extract.

Results and Discussion

The results from the present study showed that aqueous& ethanol extract of two medicinal plants displayed antibacterial activity against seven pathogenic bacteria. Results showed that diameter of inhibition zones ranging from 1-3 mm, with the highest zone observed against *E. coli* of *Lippia geminate.*

Minimal inhibitory concentration MIC: MIC of the plant extract was carried out using broth dilution method. For bacteria, nutrient broth containing different concentration (12.5–100µg/ml) of ethanol extract were prepared and inoculated with 0.1 ml inoculums. The inoculated culture were incubated at 37℃ 24 hrs. The least concentration of the extract causing complete inhibition of the growth was taken as MIC after 24 hrs incubation.

The major phytochemical constituents of interests such as flavonoids, tannin, carbohydrate, protein, phenolic compound, alkaloid and sterol are found to be present in the leaf and whole plant extract of two medicinal plants.

Table.1 Antibacterial activity of two medicinal plants by agar well diffusion method

Organisms	Diameter of Inhibition zone (mm)			
	Chenopodium album		*Lippia geminata*	
	Aqueous	Ethanol	Aqueous	Ethanol
E. coli	1.8	1.0	1.0	3.0
P.aureginosa	1.5	1.2	1.5	1.5
B. subtilis	2.5	1.0	1.0	1.5
E. faecalis	2.2	2.0	0.0	2.0
K.pneumoniae	2.0	0.0	1.0	2.7
S. typhi	2.0	2.0	1.0	2.0
S. dysenteriae	1.5	1.0	0.5	1.7

Table.2 Minimal inhibitory concentration of *Lippia geminate*

Organisms	MIC values of *Lippia geminata*									
	Concentration of *Lippia geminata extract*					Concentration of Chloramphenicol				
	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	Control µg/ml	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	Control µg/ml
E. coli	0.09	0.11	0.16	0.19	00	0.03	0.05	0.05	0.06	0.17
B. subtilis	0.06	0.09	0.11	0.16	00	0.04	0.04	0.05	0.05	0.15
S.dysenteriae	0.13	0.15	0.17	0.19	00	0.04	0.06	0.08	0.08	0.11
E. faecalis	0.11	0.13	0.16	0.17	00	0.05	0.08	0.09	0.11	0.12

OD value of blank = 0.00

Table.3 Minimal inhibitory concentration values of *Chenopodium album*

Organisms	MIC values of *Chenopodium album*									
	Concentration of *Chenopodium album* extracts					Concentration of Chloramphenicol				
	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	Control µg/ml	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	Control µg/ml
E.coli	0.02	0.06	0.07	0.07	0.10	0.03	0.05	0.04	0.04	0.07
E. faecalis	0.03	0.04	0.07	0.10	0.11	0.05	0.08	0.09	0.11	0.12
S.typhi	0.09	0.09	0.13	0.14	0.17	0.01	0.01	0.02	0.02	0.18
P.aeruginosa	0.04	0.06	0.06	0.07	0.09	0.01	0.03	0.04	0.04	0.08

OD value of blank = 0.00

Table.4 Phytochemical analysis of two medicinal plants

Phytochemicals	**Plant extracts**			
	Lippia geminata		*Chenopodium album*	
	Aqueous	Ethanol	Aqueous	Ethanol
Carbohydrate	+	+	+	+
Protein	+	+	+	+
Flavonoid	+	+	+	+
Tannin	+	+	+	+
Phenolic compound	+	+	+	+
Alkaloid	-	-	+	+
Steroid/sterol	+	+	+	+

Table.5 DPPH radical scavenging activity of ethanol extract of *Chenopodium album, Lippia geminate* (Test) and Quercetin (Standard)

Concentration (µg/ml)	Percentage inhibition of *Chenopodium album*	Percentage inhibition of *Lippia geminata*	Percentage inhibition of Quercetin
10	51.00	41.00	55.13
20	69.00	53.00	79.48
30	75.16	65.19	81.62
40	82.19	68.00	88.95
50	86.16	72.16	94.30

Figure.1 shows the antibacterial activity of *Lippia geminata* against pathogen

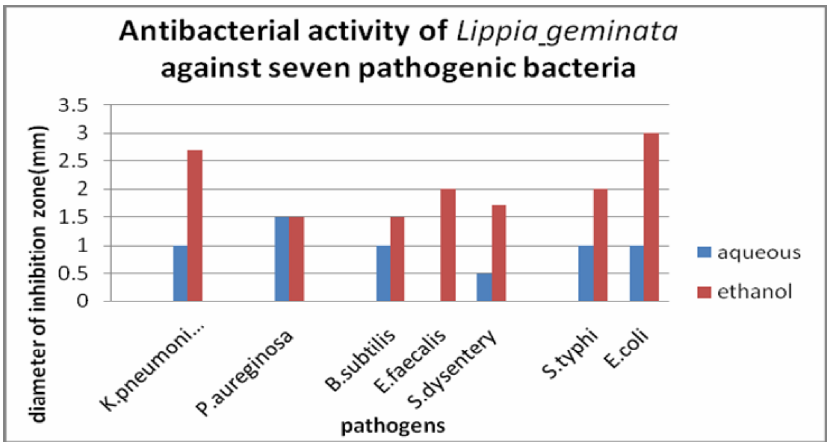
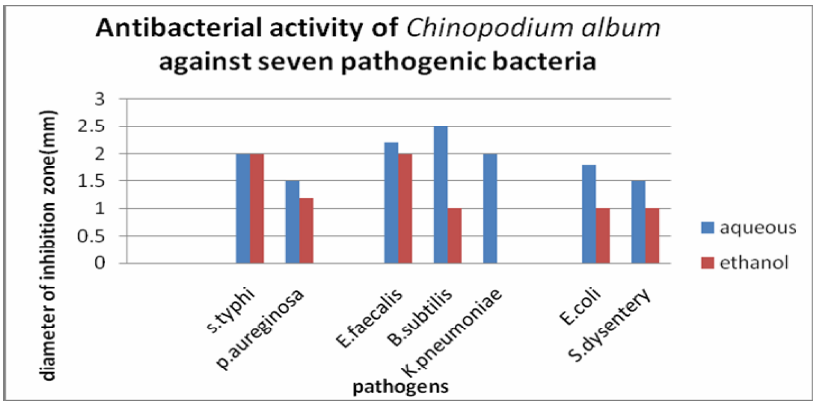


Figure.2 shows the antibacterial activity of *Chenopodium album* against pathogen



Antioxidant test: DPPH radical scavenging activity of ethanol extract of *Chenopodium album, Lippia geminate* (Test) and Quercetin (Standard) is given in table 5.

At the beginning of the 21st century the herbal medicines achieved the reliability in

mind set of global people as because it have no side effects, relatively less expensive and better patient tolerance. The plants bio-constituents have been a good source of antimicrobial agents but still many of the plant species remained unexplored. The tested two plants showed the antibacterial activity and antioxidant activity which has been represented in the table 1 and 5 and figure 1 and 2. *Lippia geminata* showed better results than *C. album*. Maximum inhibition of the extract of *Lippia geminata* found against *E. coli* (3.0mm). Considering MIC *Lippia geminata* (Table 2) in 100 µg/ml showed better results 0.06 mm against *B. subtilis*. Experimental findings revealed that ethanol extract is better than the aqueous extract to control the bacteria. We have done it in a preliminary way but we think future detail research work will find out the detail bioactive compounds within these plants and will open a new avenue to formulate and develop antibacterial compounds.

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