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In vitro Comparative Screening of Antibacterial and Antifungal Activities of Some Common Plants and Weeds Extracts

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Abstract : The Plants and Weeds are screened out for their antibacterial and antifungal activity against some gram-positive and gram-negative bacteria's by extracting out their aqueous and methanolic extracts and screened out by analyzing their zone of inhibition. The weed *Dathura stromonium* shows pronounced antibacterial and antifungal activity along with *Azadirachta indica,* when compared with some standard antibiotics.

Key words : Antibacterial, Antifungal, *Dathura stromonium*, *Azadirachta indica*

Introduction

The use of higher plants and their extracts to treat infections is an age-old practice. Traditional medicinal practice has been known for centuries in many parts of the world. Ayurveda, the science of life, prevention and longevity is the oldest and most holistic medical system available on the planet today. Herbal medicines are gaining growing interest because of their cost effective and eco-friendly attributes. In recent years much attention has been given to nonchemical systems for seed treatment to protect them against seed-borne pathogens. Plant extracts have played significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and field emergence of plant seeds.

Azadirachta indica., Neem is distributed widespread in the world. The Chemical constituents in it contain many bioactive compounds that can be extracted from neem, including alkaloids, flavonoids, tri-terpenoids, phenolic compounds, Carotenoids, steroids and ketones. Azadirachtin, a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective (Verkerk and Wright, 1993). Other compounds than azadirachtin having biological activity are salannin, volatile oils, meliantriol and nimbin. Neem leaf is effective in treating various diseases as eczema, ringworm, acne, anti-inflammatory activities, anti-hyperglycemic and also used to treat chronic wounds, diabetic foot and gangrene . It is also believed to remove toxins from the body, neutralize free radicals and as blood purifier. Recently it used as anti-cancer and it has hepato-renal protective activity and hypolipidemic effects (Ahana, 2005). Boiled neem leaf water makes an excellent antiseptic to clean wounds, soothes, swellings and eases skin problems.

Helianthus annuus, (composite), is distributed worldwide. The plant contains an oleic acid and the new triacyl glycerol was identified as isomers of oleic acid from high-saturated sunflowers. It also contains various alkaloids, glycosides, saponins, cardiac glycosides, tannins, fixed oils and simple phenolic. The Seeds have medicinal importance as diuretic, expectorant and also used for cough, throat and lung infections. It is a folk remedy for blindness, bronchitis, carbuncles, catarrh,

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colic, diarrhea, dysentery, dysuria, eyes, fever, inflammation, laryngitis, menorrhagia, pleuritis, rheumatism, scorpion stings, snakebite, splenitis, urogenital ailments, whitlow and wounds. It is also used as anticancer.

Allium cepa **(**Liliaceae), Vernacular name**:** Basal, is Distributed World wide. It contains numerous organic sulfur compounds, including trans-S-(1- propenyl) cysteine sulfoxide, S–methyl–cysteine sulfoxide, S–propylcysteine sulfoxide and cycloalliin; flavonoids; phenolic acids; sterols including cholesterol, stigma sterol, b-sitosterol; saponins; sugars and a trace of volatile oil composed mainly of sulfur compounds, including dipropyl disulfide. Onion was used for decrease cancer tumor initiated, promote healing of stomach ulcers, inhibit the proliferation of cultured ovarian, breast and colon cancer cells; reduce the cholesterol, blood pressure and symptoms associated with diabetes mellitus, inhibit platelets aggregation (involved in thrombosis) and prevent inflammatory processes associated with asthma (Augusit, 1996). Onion was used as antiseptic, antiheleminthic, antispasmodic, carminative, diuretic, cholagogge, diaphoretic and expectorant. It was used also for coughs, the flu, parasites, wound, burns, dog bites, bee stings, earaches, athletes' foot, warts, baldness, toothaches, intestinal infections, kidney infections, contaminated blood and heart failure.

If this medicinal or antimicrobial property resides in a weed that will be an added advantage. The present investigation is therefore, undertaken to test the efficacy of some of the common plant and weed extracts against the bacterial pathogens like *E. coli, Staphylococcus aureus, Klebsiella pneumoniae, and Bacillus subtilis*.

Materials and Methods

Plant materials

The three plants and some of the common weeds were collected from different parts of Meerut District, Uttar Pradesh, India and are easily identified, as they are common weeds. Table 1 shows the list of common weeds used and other plants used in this study were *Azadirachta indica* and *Helianthus annuus* and *Allium cepa*. The leaves of *Azadirachta indica*, seeds of *Helianthus annuus* and bulbs of *Allium* were air-dried, coarsely powdered and were then extracted.

Preparation of the crude extracts of plants:

Table 1 : List of Plants and weeds Selected for Anti-Microbial Activity

S. No.	Plant Name	Parts Used
1	*Lantana camera*	Leaves
2	*Parthenium hysterophorus*	Leaves
3	*Dathura stromonium*	Leaves
4	*Azadirachta indica*	Leaves
5	*Helianthus annuus*	Leaves
6	*Allium cepa*	Leaves

Hundred grams of each of the air-dried and coarsely powdered plant material was exhaustively extracted for 2 hours with petroleum ether (60-80° C) in soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure using Rota-vapor (Heidolph, Heizbad, Laborota 4001, Germany, 2000). The extracted plant material was then air-dried, repacked in the soxhlet apparatus and exhaustively extracted with methanol (98.8%) for 2 hours. The methanol extract was filtered and evaporated under reduced pressure using Rota-vapor. The extracts were dissolved in dimethyl-sulphoxide to make the final concentrations which kept in refrigerator till used. Simultaneously, water extract was prepared by adding (10 ml) of boiled distilled water to 5 gm of coarsely powdered plant, s leaves in a beaker on water bath with occasional stirring for 4 hours. The aqueous extract was then filtered and rewashed with small volume of boiled distilled water and added to the filtrate, which were then adjusted to (5 ml) volume and used immediately

Preparation of the crude extracts of Weeds

The collected plant materials were thoroughly washed and air dried.

(a) For 20% aqueous extract preparation, 2 g of plant material was crushed in 10ml. of sterile water and it was filtered using Whatman Filter Paper No.1. The filtrate was collected in sterile tube and was stored by refrigeration

(b) For 10% Ether-water solvent extraction 1:3 mixture of ether and water was prepared and 30ml of this solvent was used.

Preparation of the tested organisms

(A) Preparation of standard bacterial suspensions:

Microorganisms used

Bacterial and fungal cultures used were obtained from Subharti Medical College, Meerut.

E.coli, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae were the bacteria used and Aspergillus niger and the Candida albicans were the fungi used.

(B) Preparation of standard Bacterial and fungal suspensions:

The cultures of *E.coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* (*Aspergillus niger* (ATCC 9763), *Candida albicans* (ATCC 7596) were maintained on Saboraud dextrose agar, incubated at 25º C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100 ml) of sterile normal saline and the suspension was stored in refrigerator till used.

***In vitro* testing of extracts for antimicrobial activity**

Testing for antibacterial activity

The cup-plate agar diffusion method was adopted according to Kavanagh (1972) to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions (108-109) colony- forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 10 mm in diameter, was cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37º C for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated.

Testing for anti-fungal activity

The same method as for bacteria was adopted. Instead of nutrient agar, yeast and mould extract agar was used. The inoculated medium was incubated at 25º C for two daysfor the Candida albicans and three days for Aspergillus niger.

Standard antibiotics like Penicillin, Streptomycin, Gentamycin were also tested against each test organism. Each disc of penicillin had concentration of 10 units/disc, streptomycin – 10 µg/disc and Gentamycin – 300 µg/disc.

Results and Discussion

Studies with the aqueous and ether-water extracts of weeds and plants give varied results. Table 2 shows the results of antibacterial activity of plants and weeds extracts.

The aqueous extract of seeds of *Helianthus annuus and Parthenium hysterophorus* were found to be almost inactive against all organisms tested. The methanol extract of seeds of *Helianthus annuus* showed low activity (0.4 cm) against *E. Coli* in comparison of no activity of *Parthenium hysterophorus*, high activity (1.4 mm) against *K. Pneumonia* and inactive against the rest of tested organisms while *Parthenium hysterophorus* shows low activity against *Bacillus subtilis*. All extracts of

Table 2 : *In vitro* Antibacterial and Antifungal activity of different plants and weeds extracts

Plant Name	Type of Extract	Inhibition Zone (cm)				
		E. Coli	***B. subtilis***	***S. aureus***	***K. Pneumonia***	***A. niger***
Lantana camera	Aqueous	1.4	1.6	1.2	1.4	0.8
	Methanol	1.2	1.4	1	1.2	1
Parthenium hysterophorus	Aqueous	-	0.7	0.5	-	0.4
	Methanol	-	0.8	0.6	-	1.3
Dathura stromonium	Aqueous	1.9	2	1.6	1.7	0.9
	Methanol	1.6	1.8	1.4	1.5	1
Azadirachta indica	Aqueous	1.1	1.6	1.2	1.4	1.6
	Methanol	1.4	2.8	1.8	1.1	2.4
Helianthus annuus	Aqueous	0.4	-	-	1.4	-
	Methanol	0.9	1.4	-	-	1
Allium cepa	Aqueous	0.8	1.2	-	-	0.4
	Methanol	0.6	1.8	-	0.6	0.9

Table 1 : *In vitro* Antibacterial and Antifungal activity of different plants and weeds extracts

Antibiotic	Inhibition Zone (cm)				
	E. Coli	***B. subtilis***	***S. aureus***	***K. Pneumonia***	***A. niger***
Penicillin	2	0.7	0.8	1.5	1.3
Streptomycin	0.6	0.9	1.4	1.7	0.4
Gentamycin	0.75	2	1.8	1.3	1.2

Helianthus annuus were inactive against *S. Aureans*. The methanol extract of bulbs of *Allium cepa* exhibited high activity against *Bacillus subtilis* (2.3 cm), and A. niger (0.9 cm) and almost moderately active against others. The methanol extract of the leaves of *Azadirachta indica* exhibited pronounced activity (28mm) against *Bacillus subtilis*, high activity (18 mm) against the Gram-positive *Staphylococcus aureus*, low activity (1.1 cm) against *Escherichia coli*. The methanol extracts of the leaves of *Azadirachta indica* showed high activity (2.8-1.8 cm) against *Bacillus subtilis* and *Escherichia coli* respectively while its aqueous extract was moderately active. The aqueous and methanolic extracts of *Dathura stromonium* shows antimicrobial and antifungal activity slightly lower then that of *Azadirachta indica*, maximum (2.0 cm) activity of aqueous extract against B. subtilis have been found which shows its antibacterial activity.

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