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Evaluation of Antibacterial Activity and Phytochemical Screening of *Medicago sativa* Leaves

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Medicago sativa, Antibacterial activity, Disc diffusion assay, Minimum inhibitory concentration

A B S T R A C T

The in vitro antibacterial activity of various solvent extracts of Indian traditional medicinal plant *Medicago sativa* Leaves against clinical pathogens of human origin was evaluated. The antimicrobial activity of different solvents crude extract of plant used in traditional Indian medicine was tested by disc diffusion and turbidity assay method against three bacterial pathogens. The present screening result demonstrated that the Indian traditional medicinal plant *Medicago sativa* leaves methanol extract has potent antibacterial activity. The studied plant may be new source for novel antibacterial compound discovery for treating drugs resistant human pathogens as the results revealed that extracts exhibited a significant broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria.

Introduction

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Medicinal Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinines which have been used worldwide in traditional medicine to treat several diseases and infection. Many studies all over the world have been showed that the use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research was to evaluate the potential of plant *Medicago sativa* (family:leguminosae) and phytochemicals on standard microorganism strains Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. *Medicago sativa,*

also called as lucerene is a important perennial flowering plant in the pea family:leguminosae. It is cultivated as important forage crop in the world. *Medicago sativa* Seeds are reported to be having antifungal, anti-inflammatory properties while roots have antidiabetic and antioxidant action, flowers reported for anti-inflammatory action, plant is also reported showing antitumor and antifungal actions.

Chemical constituents of *Medicago sativa* include Calcium, carotene, chlorophyll,c owmarine derivative, choline, 8 essential amino acid, flavonols, lime, magnesium, phosphorous, protein, silicon, potassium, sterol, vitamine A, D, E, K. and iron. It also contains Saponins (2–3%) that on hydrolysis yield the aglycones medicagenic acid, soyasapogenols A, B, C, D, and E, and hederagenin and the glycones glucose, arabinose, xylose, rhamnose, galactose, and glucuronic acid; sterols (β-sitosterol, α-spinasterol, stigmasterol, cycloartenol, and campesterol, with β-sitosterol as the major component); high molecular weight alcohols (octacosanol, triacontanol); and paraffins (nonacosane, triacontane, hentriacontane). β-Sitosterol also occurs as esters with fatty acids (mainly palmitic, lauric, and myristic). Triacontanol has been shown to be a plant growth regulator that increases the growth of rice, corn, and barley as well as the yield of tomato, cucumber, and lettuce.

Flavones and isoflavones (tricin, genistein, daidzein, biochanin A, formononetin, and (−)-5′-methoxysativan); coumarin derivatives) are also reported along with Alkaloids (trigonelline, which is in seeds only; stachydrine; and homostachydrine); plant acids (malic, oxalic, malonic, maleic, and quinic, etc.); vitamins and growth factors (vitamins A, B1, B6, B12, C, E, and K1; niacin; pantothenic acid; biotin; folic acid; etc.); amino acids (valine, lysine, arginine, leucine, isoleucine, tryptophan, phenylalanine, methionine, and threonine; asparagine in high concentrations in seeds); sugars (sucrose, fructose, arabinose, xylose, galactose, ribose, mannoheptulose. The plant is also known as “alfalfa”, it can be used as antifungal, tonic, diuretic, anti-inflammatory, laxative, and hepato-protective including its usage as nutritive, digestive and detoxifier. The antifungal and antibiotic properties of alfalfa have been proved effective to cure many diseases as it contains saponins.

Alfalfa has shown some activity against tuberculosis bacteria, the basic proteins viz. histones in alfalfa display antitumor activity. In addition to these, alfalfa is further used in alleviating inflammation of the bladder, bloating or water retention, indigestion, halitosis, constipation. Apart from the substantial use of alfalfa in Herbal therapy, Naturopathy, Homeopathy and Ayurveda, the usage of this herbal plant is also popular in some tribes and folks; they use alfalfa in treating diabetes and malfunctioning of the thyroid gland. Hence, more studies pertaining to the use of *Medicago sativa* as therapeutic agents should be emphasized, especially in relation to antibacterial activity.

Materials and Methods

Plant material

The fresh and healthy leaves of the plant *Medicago sativa* collected from Ahmednagar district, Loni, cleaned and dried at room temperature in shade, away from direct sunlight and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of extract

Hot continuous extraction Technique; Soxhlet Extraction was used for sequential extraction with a series of solvents of increasing polarity i.e. Pet Ether and methanol. The marc after exhaustive pet ether extraction was air-dried and subjected to extraction with methanol (high polarity solvent). The percentage yield was calculated and the extract stored in a refrigerator at 15°C until time of use.

Phytochemical screening

Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. To perform the tests the following chemicals and reagents were used: Carbohydrates with Molisch's test, glycoside with water and sodium hydroxide solution, saponins with the capability of producing suds, steroids with chloroform and sulphuric acid, flavonoids with Mg and HCl, tannins with ferric chloride solution, gum with Molish reagents and concentrated sulfuric acid.

Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagendorff's reagent. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

Preparation of test organisms

Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were isolated in nutrient agar medium and selectively cultured at 37^0C for 24 hrs. The bacterial strains were identified by biochemical and standard antibiogram tests as per the directions from Bergy's manual for determinative bacteriology.

Antibacterial activity

Antibacterial sensitivity testing using disc diffusion method

This method depend on the diffusion of an antibiotic from vertical cylinder or cavity through the solidifide agar layer of petridish or plate to an extent such that growth of added microorganism in a cicular area is presented entirely in a cicular area or zone around the cavity. 500 µl of microbe's cultures age18 - 24 h were added to Petri plates and nutrient agar was poured. After solidifying media, holes were made by using 5 mm cork borer; each hole was filled with 50 µl of plant extract. The inoculated agar plates were left in refrigerator for one hour for proper diffusion then plates were incubated, at 37^0C for 24 h. Negative and positive controls were used. The zones of inhibition were then recorded in millimeters. (Chung, K.T. *et al*., 1998)

Turbidity assay

This method depends upon the growth of a microbial culture in a uniform solution of the antibiotic in a fluid medium that is favorable to its rapid growth in the absence of the antibiotic. Antibacterial activity of petroleum ether, methanol was tested in vitro against Escherichia coli, staphylococcu aureus and pseudomonas aeruginosa bacteria by turbidimetry method.

Determination of minimal inhibitory concentration

One ml of sterilized media was poured into sterilized test tubes. 1ml of 1000µg/ml standard solution was transferred in one tube and serially diluted to give a concentration of 333, 111,37, 12.03, 4.02, 1.2 µg/ml. the petroleum ether, methanolic extract (1mg/ml) were transferred to the test tubes. To all the tube 0.1 ml of suspension

of bacteria in saline was added and the tubes were incubated at 37° c for 24 hrs. The growth in the tubes was observed visually for turbidity and inhibition was determined for absence of growth. MIC was determined by the lowest concentration of sample that prevented the development of turbidity.

Result and Discussion

Our present investigation for the newer antibacterial bioactive compounds targeted the unexplored folk medicinal plants, being used for centuries in treating local population. The plant extracts are considered as best source of bioactive compounds particularly for traditional healers as they contain components of therapeutic values. The bioactive compounds have been detected for either bacteriostatic or bacteriocidal action. Phytochemical analysis of the pet ether and methanolic extract of *Medicago sativa* Leaves revealed the presence of lipids, carotenoids, triterpens, free sterol, alkaloids and carbohydrates. The methanolic extract found to contain tannins, glycosides and resinous substances. Hence we can say *Medicago sativa* plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The results of extracts of *Medicago sativa* Leaves, tested for antibacterial activity on three human pathogens were presented in following graph and table.

In Disc diffusion assay the zone of inhibition around the disc impregnated with plant extract over the lawn of bacterial culture plates quantitatively determined the antibacterial activity. The result showed that the antibacterial activities of plant extract were increased with increasing concentration of crude extracts and with increasing polarity of solvent used for extraction. *Medicago sativa* leaves extracts showed prominent antibacterial activity against both gram negative as well as gram positive bacteria. Among the tested plant extracts *Medicago sativa* methonalic extract showed highest activity in disc diffusion assay showing higher inhibition zone against all selected bacterias as shown in Table.1 and Graph 1. The *Medicago sativa* leaves pet ether extracts showed maximum minimum inhibitory concentration as compared with methanol extract (Table.2 and Graph 2).

Table.1 Antibacterial activity of *Medicago sativa* Leaves extract by disc diffusion method

Organism	**Zone of Inhibition (mm)**		
	Standard	**Petroleum ether extract**	**Methanol extract**
Escherichia coli	24	21	23
Pseudomonas aeruginosa	27	18	22
Staphylococcus aureus	22	19	23

Graph.1 Antibacterial activity of *Medicago sativa* Leaves extract by disc diffusion method, showing comparison of zone of inhibition

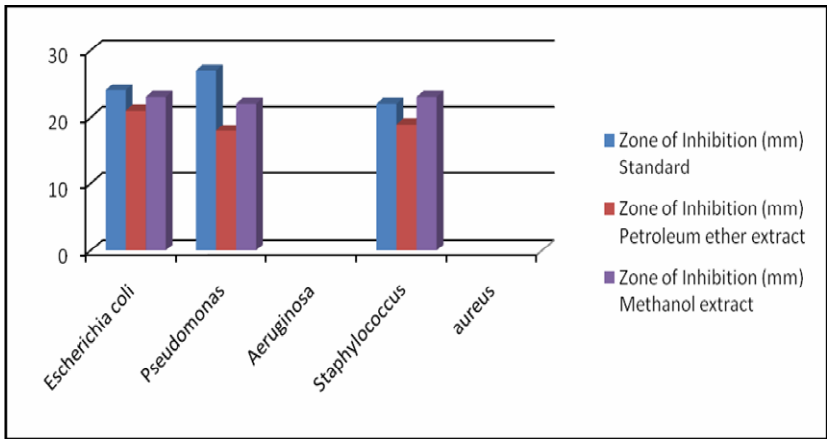
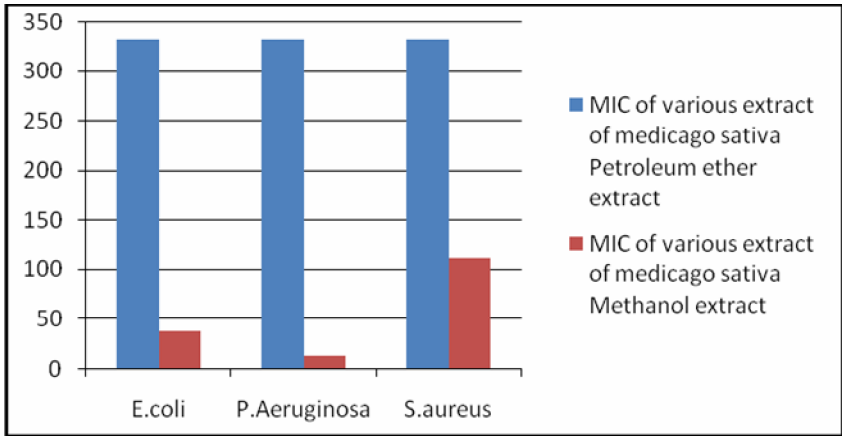


Table.2 MIC of various extract of *Medicago sativa* leaves

Organism	MIC of extracts of *Medicago sativa* leaves	
	Petroleum ether extract	Methanol extract
E.coli	333	37
P.Aeruginosa	333	12.03
S.aureus	333	111

Graph.2 MIC of various extracts of *Medicago sativa* leaves



Hence we can conclude that all extracts exhibited antibacterial activity against *E.coli. S.aureus, P.aeruginosa*. From zone of inhibition methanol extract showed prominent antibacterial activity than petroleum ether extract. From the MIC, all extract exhibited antibacterial activity against *E.coli, s.aureus, p. aeruginosa* but methanol extract showed significant antibacterial activity as compared to pet ether extract. This study serves as basis for further research on these herbs.

Conclusions

Our results allow us to conclude that the crude extracts of *Medicago sativa* leaves exhibited a significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. This probably explains the use of these plants by the indigenous peoples against a number of infections since generations. The present investigation data on antibacterial potency of *Medicago sativa* leaves will help to design further study for synthesis of novel antibiotics.

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