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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF AZADIRACHTA INDICA

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ABSTRACT

This study was carried out with an objective to investigate the antibacterial and antifungal potentials of leaves of *Azadirachta indica* Linn. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of hydroalcohol extracts of leaves of *Azadirachta indica* Linn. (an ethnomedicinal plant) was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of extracts (50, 100, 250, 500,1000 µg/ml) of *Azadirachta indica* were tested against two Gram-positive—*Staphylococcus aureus and Penicillum notatum.* two Gram-negative—*Escherichia coli, Pseudomonas aeruginosa* human pathogenic bacteria; and fungal strains—*Aspergillus niger*. Zone of inhibition of extracts were compared with that of different standards like, chloramphenicol for antibacterial activity and citokonazole for antifungal activity. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out. The microbial activity of the *Azadirachta indica*was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: , secondary metabolites, Gram-negative, Gram-negative, Ethnomedicinal.

1.INTRODUCTION

Neem is an attractive broad-leaved, evergreen tree which can grow up to 30m tall and 2.5m ingirth. Its trunk usually straight is 30-80 cm in diameter. Its spreading branches form arounded crown of deep-green leaves and honey-scented flowers as much as 20m across.The composition of neem cake after the extraction of oil varies widely depending on the rawmaterial used for expelling, for example, whole dried fruits, seeds or kernels. The range ofthe proximate composition in percentage are: crude protein 13-35, carbohydrates 26-50,crude fibre 8-26, fat 2-13, ash 5-18, acid insoluble ash 1-7. The bitter cake has no value asanimal or poultry feed. Extraction of cake with 70% alcohol followed by hexane yields ameal free from bitterness and odour, which will be satisfactory as feed. The neem cake is richin most of the amino acids. It is a potential source of organic manure and contains many plantnutrients, viz., nitrogen 2-3%,

phosphorus 1% and potassium 1.4%. It also contains 1.0-1.5%tannic acid and has the highest sulphur content of 1.07 - 1.36% among the oil cakes. Theneem cake contains a large number of triterpenoids, more of which are being discovered.

Neem - the legendary medicinal tree of India, has grown with the human settlement allover the country and has been an integral part of the Indian way of life for centuries. Thehistory of the Neem tree is inextricably linked to the history of the Indian civilization.The Neem tree has for a very long time been a friend and protector of the Indian villager.For ages Indians have trusted this tree to fortify their health and remedy scores of diseases.In addition, it has been used for protecting food and stored grains and as a fertilizer andnatural pesticide for the fields. It has been used for a far wider array of uses than any othertree.[1-5]

Nimibidin found in neem bark is now known to be antipyretic and non-irritant, and it hasfound to be effective in treatment of skin diseases such as eczema, furunculosis, arsenicaldermatitis, burn ulcers, Herpes labialis, scabies and seborrhaeic dermatitis.It is also effective in the treatment of skin diseases of unknown origin, such as warts anddandruff.Extracts of bark have potent diuretic and anti-inflammatory properties.Nimbidin and sodium nimbidinate contained in neem bark are reported to possess spermicidalactivity. Neem bark has shown anti-bacterial activity against various gram positiveorganisms.[5-8]

- The present study aims to give a detailed description of leaf extract of *Azadirachta indica.*
- The main objective of the study is to evaluate the antimicrobial activity of the Ethanol and chloroform extract of *Azadirachta indica.*.
- This antimicrobial activity of the Ethanol and chloroform extract is compared with that of the standard Ketoconazole and Chloramphenicol.

2.MATERIALS AND METHODS

The experimental procedure employed in the present investigation was to evaluate the various parts of *Azadirachta indica* for their antimicrobial and property.

COLLECTION OF PLANT MATERIAL

Azadirachta indica(leaves) were collectedfrom the local area. The collected leaves, stem and root of *Acalypha indica* were washed with tap water, then surface sterilized with 10 per cent sodium hypochlorite solution, raised with sterile distilled water and allowed to shade dried under room temperature. The samples were ground into fine powder using an electric blender.

PREPARATION OF EXTRACT

One hundred grams of each powdered plant material was successively extracted with, chloroform, and Ethanol by using Maceration process for 24 hours .

The extracts were filtered, pooled and the solvents were evaporated with the help of rotary evaporator (Remi, Germany) under reducedpressure at 40□C and the crude extracts were kept at

4□C in refrigeratorfor antimicrobial assay.

General laboratory techniques for the preparation of the media, inoculation and maintenance of culture are as follows.

All the glassware were kept in chromic acid cleaning solution (10 g of potassium dichromate in 1000 mL of 36 N sulphuric acid) for four hours. The glassware were washed thoroughly with detergent solution using tap water and then rinsed with distilled water. Then the glassware were dried in dust proof cupboard and oven dried at160^0C for 1 h for further use.[9-10]

Sterilization Media were sterilized in an autoclave at 15 lbf/sq inch pressure for 30 min. The glassware was sterilized with the help of hotair oven at 120□C for an hour. Hot air oven was used to sterilize all glass syringes and test tubes (should be plugged with cotton wool). Inoculation loops, points of forceps were sterilized with Sprit lamp till they become red hot for sterilizing them. Scalpels, needles, mouth of culture tubes *etc.,* were passed through the Sprit lamp for few minutes without allowing them to become red hot and sometimes they were immersed in Ethanol spirit burnt off.

Chemicals

The chemicals, organic solvents and the reagents used in the study were of analytical reagent (AR) quality and the media were obtained from Mahaveer Ltd.

ANTI BACTERIAL ACTIVITY:

Agar well diffusion method:

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [36-37]. Similarly to the procedure used in disk-diffusion method, Nutrient agar medium was prepared and autoclaved at 121^{o}C, 10 lb pressure for 20 mins. Allow the media to cool, before the media gets solidifythe agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, 50µl of the Ethylacetate Extract and Silver nanoparticles (10mg/ml in DMSO) were loaded into the wells as per the labeling and incubated at 25^{o}C for 4-5 days. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.The plates were observed for zone of inhibition every day up to 5 days and measured the zone of inhibition.

Nutrient Agar Medium: Nutrient agar medium is used as a general purpose culture medium which may be used as enriched medium by incorporating blood or other biological fluids in accordance with Indian Pharmacopoeia.[11-15]

Directions

Suspend 37.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10lbs pressure (115°C) for 30 minutes or alternatively at 15 lb pressure (121°C) for 15 minutes or as per validated cycle.

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Principle and Interpretation

Nutrient Agar is a basic culture medium used for maintaining microorganisms [34], for purity checking prior to biochemical or serological testing. It is used for the cultivation and enumeration of bacteria, which are not particularly fastidious. In semisolid form it is used for maintenance of control or standard organisms. Indian Pharmacopoeia has recommended it for microbial limit tests of viable aerobic microorganism present in pharmaceutical substances [35]. Peptone and meat extract B provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients.Sodium chloride maintains osmotic equilibrium. Nutrient media may be used as enriched media by the addition of 10% v/v blood or other biological fluids like ascitic fluid, serum etc.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel

Colour and Clarity of prepared medium: Light yellow coloured clear to slightly opalescent gel forms in Petri plates

P^H: 7.20-7.40

Growth Promotion Test: Growth Promotion is carried out as per Indian Pharmacopoeia

Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Storage and Shelf Life: Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry date on the label.[16-19]

3.RESULTS

ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *AZADIRACHTA INDICA*

The present investigation, deals with the antibacterial, antifungal activity as well as phytochemical screening of *Azadirachta indica*. The plant extracts were prepared from the leaves of *Azadirachta indica*by using the solvents of different polarity such as chloroform and Ethanol by Maceration. The extracts were evaluated for antibacterial activities.

Bacteria:Gram-negative clinical isolates viz., Escherichia coli, Pseudomonas aeruginosa,Proteus vulgaris, Staphalococcus aureus, Bascillus subtilus, And Klebseliea species.

PHYTOCHEMICAL SCREENING:

TEST FOR ALKALOIDS:

TEST	OBSERVATION	INFERENCE
1)Dragendroff's test: Dragendroff reagent+ Sample	Reddish brown ppt	Alkaloids are present

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2)Mayer's test: Mayers reagent + Sample	Cream colour ppt	Alkaloids are present
3)Wagners'test: Wagners reagent + Sample	Brown colour ppt	Alkaloids are present
4)Hager's test: Hagers reagent + Sample	Yellow colour ppt	Alkaloids are present

DETECTION OF TANNINS

TEST	OBSERVATION	INFERENCE
Ferric chloride test: 1 drop of $Fecl_3$+ sample	bluish or greenish black coloration	indicate the presence tannins.
Gelatin test: 1% gelatine solution + Nacl+ sample	white precipitate was observed	indicate the presence tannins.

Zone of inhibition

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4. DISCUSSION

Medicinal plants are considered to be storehouses of potential antimicrobial crude drugs as well as sources for novel compounds with antimicrobial activity, with possibly new modes of action. Some naturally occurring plant compounds can kill antibiotic-resistant bacterial strains *viz., penicillum notatum, Aspergillus niger, proteus vulgaris, Bacillus subtillus, Escherichia coli* and *Staphylococcus aureus*

Researchers are increasingly turning their attention to the medicinal plants and they estimated that plant materials are present in or have provided the models for 25-50 per cent western drugs. Many commercially proven drugs used in modern medicine was initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activities. Plant derived medicines are relatively safe than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment

In the present research,the *Azadirachta indica*(leaves) were subjected to antimicrobial activity

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against *Staphylococcus aureus, klebsiella species, bascillus subtillus,Escherichia coli,Pseudomonas aeruginosa, Proteus vulgaris*,,and *Aspergillus niger* and *pencilliun notatum* also thepreliminary phytochemical analyses were carried out.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *AZADIRACHTA INDICA*

ANTIBACTERIAL ACTIVITY:

In the present study, the various solvents like chloroform, Ethanol extracts of the leaves of *Azadirachta indica*were screened against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus sutilus, klebsiella species.*The antibacterial activity of leaf extracts of *Acalypha indica* against different microorganisms like *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus sutilus, klebsiella species.* And the anti bacterial activity of leaf extracts of *Acalypha indica* was not found. So there is no anti bacterial activity of leaf extracts of *Acalypha indica.*

STANDARD DRUG FOR ANTIBACTERIAL ACTIVITY:

The comparative study of antibacterial was done with chloromphenicol standard drug against different micro organisms like, Bacillus subtilis, Escherichia coli, proteus vulgaris, klebsiella, pseudomonas aeruginosa, staphyloccus aereus. The diameter of zone of inhibition was found to be, Pseudomonas aeruginosa(2.4),
Bacillus subtillis(2.0),
Escherichia coli(1.79),
Proteus vulgaris(2.11),
Staphylococcus aereus(2.10),
klebseialla species(2.69).

PHYTOCHEMICAL ANALYSES

In the present study, the phytochemical constituents of leaves of *Azadirachta indica*(which were extracted using different solvents *viz.*(chloroform, ethanol) were analysed. The chloroform and ethanol extracts of leaves of *Azadirachta indica*contained phytochemicals such as alkaloids, flavonoids, tannins and saponins than the other solvent extract (water).

Previous reports proved that *Azadirachta indica*have exhibited different kinds of secondary metabolites. The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these include: alkaloids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement and body building (Kubmarawa *et al*., 2008). A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. It is due to the presence of non-nutritive plant phytochemicals. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, the phenolics such as flavonoids and tannins are the group of compounds that act asprimary antioxidant or free radical scavengers

Several research studies have demonstrated that herbal plants contain diverse classes of compounds such as polyphenols, alkaloids, tannins and carotenoids (Zheng and Wang, 2001). Alkaloids have been well investigated for many pharmacological properties including antiprotozoal, cytotoxic, antidiabetic and anti-inflammatory properties but there are only few reports about their antimicrobial properties.

Flavonoids are referred as natures biological response modifiers, because of their inherent ability to modify the body's reaction to allergies, anti-inflammatory, antimicrobial, anticancer activities. Apart from this flavonoids show potent antihyperglycemic activity in animal studies

Tannins were reported to exhibit antidiabetic, anti-inflammatory antibacterial and antitumor activities. It has also been reported that certain tannins were able to inhibit HIV replication selectively besides use as diuretics. Plant tannins have been widely recognized for their pharmacological properties.

Saponins are glycosides occurring widely in plants. They are abundant in many foods. In medicine, it is used in hypercholesterolemia, hyperglycemia antioxidant, anti-cancer.

5.CONCLUSION

In the present study of ethanol extract of *Azadirachta indica*leaves has shown very good antibacterial activity. However, more clinical and pathological studies must be conducted to investigate the unexploited potential of the plant.

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