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A Review on Extraction of Neem Leaves and Study the Antimicrobial Activity

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ABSTRACT: The purpose of present study was to investigate the antimicrobial activity of neem leaves against human pathogenic bacteria including E. coli, Pseudomonas aeruginosa, Salmonella typhimurium,Staphylococcus aureus, Bacillus pumilus. A. indica belongs to the family Meliaceae, commonly known as neem. It is used in traditional medicine as a source of many therapeutic agents. A. indica (leaf, bark and seed) are known to contain antibacterial, antifungal, activities against different pathogenic microorganism and antiviral activity against vaccina, chikungunya. Different part of neem have been shown to exhibit wide pharmacologic activities including; antioxidant, antimalarial, antimutagenic, anticarcinogenic, antiulcer, anti-inflammatory, antidiabetic properties.

KEY WORDS: Azadirachta indica, E.coli, Pseudomonas aeruginosa ,Salmonella typhimurium, Bacillus pumilus.

I.INTRODUCTION

[1]Antimicrobial in leaf extract of neem (Azadirachta indica) against human pathogenic bacteria E.coli, Staphylococcus aureus , Pseudomonas aeurginosa , Salmonella typhimurium, Bacillus pumilus. Antimicrobial activities of alcoholic extract of neem leaves were used. Varying concentration of each extracts 200mg\ml 150mg\ml 100mg\ml 50mg\ml,25mg\ml prepared by using disc diffusion method. When compare to gentamycin 200mg gentamycin 10mg, the methanol and ethanol extract shows maximum inhibition on bacillus pumilus , pseudomonas aeruginosa and staphylococcus aureus in an ascending order. Neem is used as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. Neem have showed that it contains active substances with multiple medicinal properties. Aqueous extract of neem leaf extract has a good therapeutic potential as anti hyperglycemic agents. Neem leaf extract also have anti-inflammatory properties .

[2]Neem leaves has antibacterial properties and could be used for controlling air bone bacterial contamination in the residential premise. Neem seeds in traditional medicines is used to treat infection conditions especially those involving in the eye and ear. Administration of alcoholic extract of neem flower disrupts the estrous cycle in Sprague Dawley rats and causes partial block in ovulation and has the potential of an ideal antifertility agents. The great potential neem aqueous extract as powerful chemotherapeutic and viral agent

II.PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACT OF AZADIRACHTA INDICA LEAVES

[3]Azadirachta indica commonly known as neem is a fast growing tropical evergreen tree found mainly in India, Africa and America. Neem is called by various names in India such as “divine tree”, “wonder tree”,” heal all”, “nature’s drug store”, “ village pharmacy”. All part of neem tree-leaves, flowers, seed, fruits, bark, root have been used traditionally for the treatment of inflammation, infection, fever, skin diseases and dental disorders. Neem leaf and it’s constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antimalarial, antibacterial, antiviral, antioxidant and anticarcinogenic properties

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III.MATERIALS AND METHOD

PREPARATION OF EXTRACT

[2]Neem decoction (Distilled water): Fresh Azadirachta indica leaves were collected and washed under running tap water and rinsed with distilled water to remove dust and debris. The leaves were crushed In a mortar and pestle to a coarser particle size. The crushed leaves(50gm) added with 40ml of distilled water and boiled till the volume reduces to 20 ml. Decoction was passed through muslin cloth and allowed to cool down at room temperature. The prepared decoction was passed through muslin cloth and filtrate used for further research study**.**

Neem juice:Fresh Azadirachta indica leaves were collected and washed under running tap water and rinsed with distilled water to remove dust and debris. The neem leaves were crushed in a mortar and pestle with a little quantity of distilled water to obtain a juice.These crushed leaves were passed through the muslin cloth. And the obtained filtrate was used for further research study.

Aqueousextract:The collected Azadirachta indica leaves were washed under running tap water and rinsed with sterile distilled water to remove a dust and debris. The leaves were separated out from each after wash, segregated and shed dried. The shed dried leaves were powdered using a dry mechanical grinder. The dried powder was passed through the mesh sieve to obtain the fine powder. The fifty gram of leaves powder was added with 250 ml of distilled water. This conical flask was stoppered tightly and was kept at room temperature for maceration of 48 hours and during maceration period the content of conical flask was shaken at interval of two hours. Content of the conical flask was filtered through a muslin cloth. The content left in the flask was rinsed twicely by taking the little quantity of the filtrate so obtained was once again filtered through Whatman number 1 filter paper. The filtrate was transferred to sterilized evaporating bowl and kept under fan for evaporation of the solvent. After complete evaporation of the solvent the extract left in the bowl was taken in the airtight screw cap vials and stored in refrigerator for use as per the requirement in experimental investigation.

VI.QUALITATIVE PHYTOCHEMICAL ANALYSIS

[4]All extracts of Azadirachta indica leaves were subjected to qualitative phytochemical analysis to identify presence of various phytochemicals viz. alkaloids, glycosides, proteins, Reducing sugar, tannins, resins, sterols, phenolic Compounds.

1. TEST FOR DETECTION OF ALKALOIDS:

A small amount of extract was taken in test tube and added With 5 ml of 1.5 % HCl (v/v) and then filtered. A few drops Of each of the following reagents were added to the filtrate and mixed well, appearance of turbidity or any changes in colour to the test indicates the presence of alkaloids.

(a)Dragendroff's reagent test:Dragendroff's reagent: It was prepared by mixing solution -A comprising 17 g of bismuth sub nitrate and 200 g of tartaric acid added into 800 ml of distilled water and Solution- B 160 g of potassium iodide in to the 400 ml of distilled water. Both, the solution A and B were mixed in 1: 1 proportion volume by volume. From this working standard was prepared by taking 50 ml of this solution, added with 100 g of tartaric acid to make volume up to 500 ml with distilled water. The solvent extract (filtrate) was sprayed on a filter paper using chromatographic sprayer and was dried. The reagent was applied on above prepared filter paper using capillary tube, the development of orange to red color confirmed for the presence of alkaloid. The solvent extract (filtrate) was sprayed on a filter paper using chromatographic sprayer and was dried. The reagent was applied on above prepared filter paper using capillary tube, the development of orange to red colour confirmed.

(b) Wagner's reagent test: The reagent was prepared by dissolving iodine 1.27 G and 2 G of potassium iodide in 5 ml of distilled water and diluted to 100 ml. The little amount of the above extract (filtrate) was added to this reagent, appearance of brown to flocculent precipitation revealed the presence of alkaloid.

2. TEST FOR DETECTION OF GLYCOSIDE:

(a)Benedict's reagent test:Equal quantity of both the extract and benedict's reagent was added and heated to boil for two minutes, appearance of brownish to red colour indicate presence of glycoside.

(b)Folic Wu copper reagent test:A little amount of extract was added to few drops of folic Wu copper reagent, the development of red colour gives positive reaction for glycoside.

3. TEST FOR DETECTION OF PROTEINS:

(a)Xanthopterin test: A small amount of the extract was added with 0.5 ml of Concentrated HNO3, the appearance of white or yellow Precipitate indicates the presence of proteins.

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(a)Biuret test:Few amounts of the extracts were added to 4% sodium hydroxide solution followed by a drop of 1% copper sulphate solution, the development of violet to pink colour indicates the presence of protein.

4. TEST FOR DETECTION OF REDUCING SUGAR:

(a)Benedict's reagent test:The extract was added with benedict's reagent in equal Amount and mixture was heated for 2 minutes, Appearance of brown to red colour indicates presence of Reducing sugar.

(a)Folin copper reagent test:Few quantities of the extract were added with few drops of folin Wu copper reagent, the development of red colour Indicates presence of reducing sugar.

5. TEST FOR DETECTION OF TANNINS:

A little quantity of alcohol extract taken in a test tube was Warmed and filtered. The filtrate was used to carry out the Tests.

(a)Lead acetate test:Few drops of 5% lead acetate solution were added to the Filtrate, formation of precipitation indicates the presence Of tannins.

(b)Ferric chloride test: Few drops of ferric chloride were added to the little amount of the filtrate, development of green color indicates the presence of tannins.

6. TEST FOR DETECTION OF PHYTOSTEROLS:

(a)Salkowski reaction : A small amount of extract was added with 2 ml of concentrated H2SO4 and was shaken for few minutes and mixed well, the development of red or brown colour indicates the presence of sterols.

7. TEST FOR DETECTION OF PHENOLIC COMPOUNDS:

A small amount of extract was treated with 2 ml of ferric chloride solution and shaken for few minutes. The appearance of pale brown colour to the test indicates presence of phenolic compounds.

8. Test for Saponins:

(a)Foam test: A small amount of extract was treated with 2ml of sodium bi-carbonate and added with distilled water, the mixture shaken vigorously. The development of froth to the test indicates the presence of saponins.

V.EXTRACTION OF NEEM LEAF

[2]Methanolic Extract:Mature plants of Azadirachta indica were used for the extraction method.

Leaf Methanolic Extract: The completely dried leaves Were coarsely powdered and 50 g was used for successive Extraction in 250 ml methanol for three days with periodic Shaking. Then, the extract was filtrated and the filtrate was Collected. The filtered liquid extracts were subjected to Rotary evaporation and subsequently concentrated under reduced pressure (in vacuum at 40°C). Then, the extracts Were evaporated to dryness and stored at 4°C in an air tight bottle

Microorganism:Gram-positive strains of Staphylococcus Aureus , Bacillus subtilis ,Bacillus cereus, clostridium perfringens, Listeria monocytogenes and Micrococcus luteus and Gram-negative Strains of Salmonella typhimurium and Escherichia coli , were used

Medium: Peptone water, Muller Hinton Broth and Muller Hinton agar were used for the preparation of the inoculum, Preservation and disc diffusion agar for the bacterial Strains, respectively.

Inoculum Preparation: Each strain was inoculated in peptone medium and incubated at 37° C for 3-4 hours and The resulting bacteria were used as inoculums. A sterile Cotton swab was dipped into the bacterial suspension and then rotated and pressed on the wall of the test tube to Get rid of excess fluid. The surface of a Muller Hinton Agar plate was inoculated with the bacterial strain.

Agar Well-Diffusion Method for Screening the of Neem Methanolic Extract:To determine the antimicrobial activity of neem methanolic Extract, the agar well-diffusion method was used. Muller Hinton agar plates were swabbed using a cotton swab from an 8 hour-old broth culture of Gram-positive or Gram-Negative bacteria. By using a cork borer, wells (8-mm Diameter and approximately 2-cm apart) were made in each of these plates. A stock solution of each neem methanolic Extract was prepared at a concentration of 1 mg/ml in methanol. Approximately 100 µl of neem methanolic extract was added into the wells and allowed to diffuse at room temperature for 2 hrs. A control well comprising methanol without plant extract was also made. The plates Were incubated at 37°C for 18-24 h for bacterial pathogens. The zone of inhibition (marked as either positive (+) or Negative (-)) was used as indicator for the effect of the Extract against bacterial species overnight cultures of the Gram-positive strains Staphylococcus aureus, B. subtilis, B. cereus, clostridium Perfringens, Listeria monocytogenes and Micrococcus and the Gram-negative strains Salmonella Typhimurium and E.

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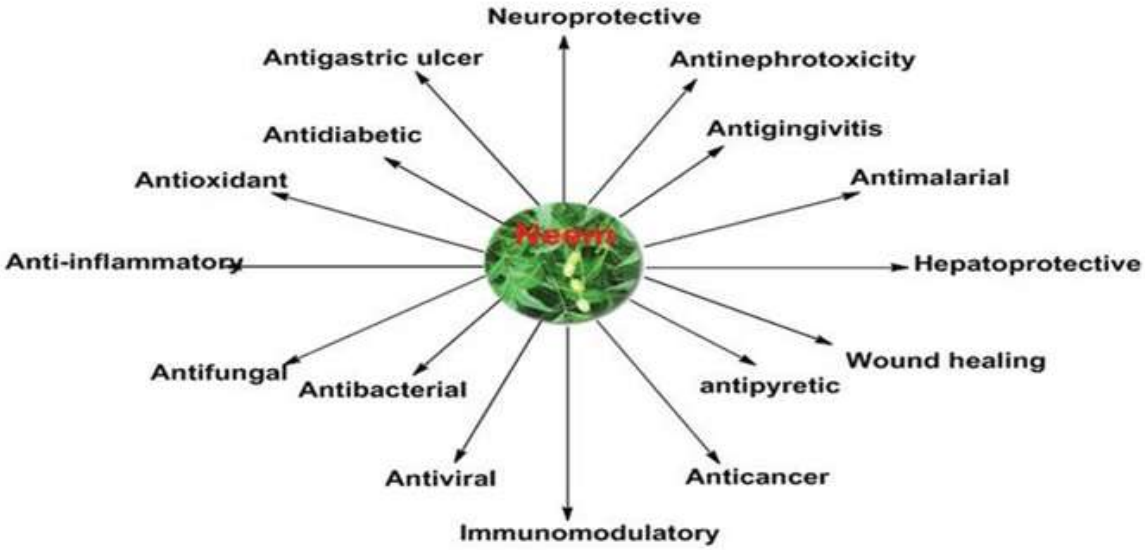
coli were suspended in Ringer's solution to a turbidity equivalent of 0.5 McFarland (1.5 ×10 CFU/ml) and 100 µl was spread onto Mueller-Hinton agar plates.

Neem Extract Preparation: Neem leaf was dried in an Oven at 50°C and prepared by blending 50 g of the dried leaf with 100 ml of methanol for 10 min. The crude extract was filtered through muslin followed by Whatman No. 1filter paper prior to autoclaving (121°C for 15 mins) before storage at -20°C.

Polyacrylamide Gel Electrophoresis (PAGE): The Preparation of isolates for SDS-PAGE and the running of the samples were performed as follows: Electrophoresis was performed in a 12% polyacrylamide running gel and A 4% stacking gel, with a 0.025 M Tris, 0.19 M glycine buffer (pH 8.3) and 100 µL of sucrose buffer (50 mM Tris–HCl, pH 8; 40 mM EDTA, pH 8; 0.75 M sucrose).

VI.SCREENING FOR BIOACTIVITY OF NEEM

[5]The extracts of different parts of neem tree (Azadirachta indica) have been well documented for its pharmacological or medicinal properties and their wide applications by indigenous healthcare practices. Pharmacological properties exhibited the remarkable biological activities that could be further explored for development of new herbal formulations and therapeutic agents. Pharmacological properties of neem tree are due to the presence of active phytochemicals like flavonoids, terpenoids, coumarins, alkaloids, tannins, sulphurous compounds, carbohydrates, proteins and minerals. Various medicinal properties and applications of neem tree have been well documented in ancient Indian system of medicine and scripts such as Susruta Samhita and Charak Samhita. Over 700 herbal preparations based on A. indica have been recognized in traditional system of medicine such as Unani, homoeopathy, Ayurveda and Siddha, and more than 160 local practices are known in different countries of the world in which neem contributes as a main or the sole constituent for curing various diseases. Neem displays various medicinal properties such as antioxidant, anti-inflammatory, antidiabetic, anticancer, antiviral, antibacterial, antigingivitis, antifungal, antiulcer, hepatoprotective, neuroprotective, antipyretic and wound healing activities. All the parts of neem tree have been used as traditional medicines. In addition to its therapeutic potential, neem is being extensively used as eco-friendly commercial agrochemicals and pesticides. The present chapter provides the critical description of phytochemistry and pharmacological properties of different parts and important natural bioactive compounds isolated from neem tree.



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VII.CONCLUSION

Many of the existing synthetic drug cause various side effects. Hence drug development plant based compounds could be useful in meeting his demand for newer drugs with minimal side effects. A. indica leaves possessed good antibacterial activity , confirming the great potential of bioactive compounds and useful for rationalizing the use o this plant in primary health care. The extract of neem when used as medicinal plant, could be useful for the growth inhibition of carcinogenic bacteria. The phytoconstituents alkaloids, flavonoids, glycosides, saponins are antibiotic principle of plants. These antibiotic principle are actually the defensivemechanism of the plant against different pathogens.

In this study shows that the methanolic extract of A. indica leaves exhibit antibacterial activity against all tested organism with concentration depended. Based on the finding in this study , it concluded that, the leaves of extract of A. indica has a potent antibacterial activity against various strain of bacterial pathogens ranges from gram positive to gram negative bacteria as such owing to its versatile characteristics neem is rightly called the 'Village pharmacy' or 'Doctor tree' or 'The bitter gem'.

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