PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF NEEM (Azadirachta indica)

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Abstract

Azadirachta indica (neem) is a plant which has been used for a long time as traditional medicine for household remedy against various human ailments from antiquity. As microbial pathogens developing resistance against current drug formulation so there is always a continuous need to isolate a new drug from plant sources. Keeping this in mind the present work was designed to identify antimicrobial potential of photochemical present in neem leaves. In present study the phytochemical screening of neem leaves for alkaloids, carbohydrates, flavonoids, glycosides, phenols,resins, saponin, steroid, tannin and terpenoids was performed. The result revealed the presence of alkaloids, cardiac glucosides, flavonoids, phenols, resins, saponins, tannins, terpenes and steroids in methanolic leaf extracts.The antimicrobial analysis of neem extract showed highest activity against *Staphylococcus aureus*.

Keywords: Alkaloids, *Azadirachta indica*, Phytochemicals, *Staphylococcus aureus*.

I. INTRODUCTION

Neem (Azadirachta indica) belongs to the family Maliaceae (Margaret, 1965). The plant has been used for a long time in agriculture and medicine (Natarajan et al., 2003). It provide best alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. The medicinal properties of the plant were studied by several workers. The antipyretic effect, antimalaria effect, antidiabetic effect, antifertility effect effect on the central nervous system cardiovascular effect and wound healing were some of the studies of earlier workers. (Thompson and Anderson, 1978, Sinha et al., 1984, Phillai and Shanthakumari, 1984, Jayaprakasan et al.,2014). Oils from the leaves, seeds and bark show antibacterial action against number of bacteria (Khan and Wassilar, 1987). The present investigation was performed to isolate phytochemicals from leaf extract and to study antibacterial activity of extract.

II. MATERIALS AND METHODS

Neem leaves were collected from local area and were washed under tap water and distilled water. 10 g of leaf powder was used for solvent extraction via Soxhlet apparatus with methanol. The extract was evaporated at room temperature. Phytochemical screening was carried out to determine the presence of saponins, tannins, flavonoids, glycosides, terpenoids, phytosterols, resins and cardiac glycosides, proteins, carbohydrates and phenols.

Phytochemical Screening

1. Test for Saponins (Foam test)

To test presence of saponins 200 mg of sample powder was mixed with 5 ml of distilled water and was shaken vigorously for a stable persistent broth. Foam formation confirmed the presence of saponins.

2. Test for Tannins (Ferric chloride test)

Few drops of 0.1% ferric chloride solution was added in neem extract. Formation of blue black colour indicated the presence of tannins in extract.

3. Test for Alkaloids (Wagner's test)

2-3 drops of Wagner's reagent was added in 0.5ml turmeric extract. Formation of reddish brown precipitate indicated the presence of alkaloids.

4. Test for Flavonoids (Alkaline reagent test)

Few drops of sodium hydroxide were added in the extract solution. First formation of an intense yellow color and then turning in to colourless solution on addition of few drops of dilute acetic acid indicate the presence of flavanoids in extract.

5. Test for Sterols and Triterpenoids (Salkolwski's test)

After treating with chloroform, few drops of concentrated H_2SO_4 was added in extract, the test tube will be shaken well and allowed to stand for some time. The appearance of red colour in upper layer confirmed the presence of sterol and formation of yellow colour at the lower layer confirmed the presence of triterpenoids.

6. Test for Cardiac Glycosides (Keller Killani test)

After drying with chloroform, 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride solution was added in extract. 0.5 ml of concentrated H_2SO_4 was added along the sides of the test tube. Blue color formation in acetic acid layer indicated the presence of cardiac glycosides.

7. Screening of Phenol

1ml of the extract was treated with 3ml 10% lead acetate solution. A bulky white precipitate indicates the presence of phenolics in extract.

8. **Screening for Carbohydrate test**

1ml of extract was treated with 1ml of Benedict's reagent. The mixture was heated on a boiling water bath for 2 minutes solution. Appearance of green color showed the presence of reducing sugar in extract.

9. Screening for Resins

Two milliliters of acetic anhydride was added to 2.0ml of the extract and a drop of concentrated sulphuric acid was also added. The observation of a purple colour, rapidly changing to violet indicates the presence of resins

Bacterial Cultures

1. *Escherichia coli* (NCIM-2064)
2. *Pseudomonas aeruginosa* (NCIM-5210)
3. *Staphylococcus aureus* (NCIM-2079)

Agar Well Diffusion Method

Anti-bacterial potential of neem leaf extract was tested by Agar well diffusion method. Nutrient agar was autoclaved and poured in the Petri plates under laminar air flow. After solidification of media the bacterial suspension (24 hrs old) was spread over the media. The wells were prepared using cork borer. Extract was dissolved in DMSO (Di Methyl Sulfoxide) in different concentrations such as 25, 50, 100 µg/ml. 40 µl test sample from each concentration was loaded to the wells and incubated for 24 hrs at 37ºC. DMSO was used as a negative control whereas amoxicillin antibiotic disc (10µg) was used as positive control.

III. RESULTS AND DISCUSSION

The result revealed the presence of alkaloids, cardiac glucosides, flavonoids, phenols, resins, saponins, tannins, terpenes and steroids in methanolic leaf extracts (Table-1). In table -2 antimicrobial activity of neem leaf extract was studied. Results showed that *Staphylococcus aureus* was highly sensitive to methanolic extract. Antimicrobial activity of extract increases as the concentration increases. *Escherichia coli* showed no sensitivity against extract. The data supports the hypothesis that

neem has an inhibitory effect on the growth of certain pathogens due to presence of phytochemicals. Then, further *in vivo* studies will certify their physiologic role.

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Table 1: Phytochemical analysis of Plant extract (in methanol)

Name of the Phytochemical Constituents	Methanolic Extract of Neem
ALKALOIDS	+ve
CARBOHYDRATES	+ve
FALAVONOIDS	+ve
GLYCOSIDES	+ve
PHENOLS	+ve
RESINS	+ve
SAPONIN	+ve
STEROID	+ve
TANNINS	+ve
TERPENOIDS	+ve

+ve: Indicates the presence and -ve: Indicates the absence of phytochemical

Table 2: Effect of neem leaf extract on growth of bacteria *in vitro*.

Bacteria	Methanol extract(µg/ml)			DMSO (Negative control)	Amoxycillin (Positive control)
	25	50	100		
Pseudomonas aeruginosa	-	7	11	-	18
Staphylococcus aureus	-	9	14	-	21
Escherichia coli	-	-	-	-	15