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Original Research Article

Phytochemical analysis of *Azadirachta* indica leaves

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Abstract

Azadirachta extract from the leaves of the tree has been reported to have strong biological activities against insect pests. The plant leaves were effective against all the tested organisms. Qualitative phytochemical analysis was performed for the detection of alkaloids, flavonoids, saponin, tannins and glucoside. Extraction of leaves was carried out in 500ml EtOH at 60^0C for 8 hours using the soxlet extractor, and then Extract was concentrated and stored at room temperature. Thin layer chromatography was also performed by using different solvent system for the analysis of Tanins, alkaloids, flavonoids present in plant extract. The active components separated through TLC were subjected to antimicrobial activity against the pathogens. The present study will be successful in identifying the plant with different antimicrobial activity which could be further exploited for isolation and characterization of the novel phytochemical treatment of infectious ailments. The wide use of azadirachta plant is attributable to the presence of these bioactive compounds, which may explain its many traditional uses against various ailments.

Key Words: Phytochemical, antimicrobial and qualitative analysis etc.

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INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization *Azadirachta indica* A. Juss (syn. *Melia azadirachta*) is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. The sanskrit name of the neem tree is 'Arishtha' meaning 'reliever of sickness' and hence is considered as'Sarbaroganibarini'. The tree is still regarded as 'village dispensary' in India. The neem tree has been described *A. indica* as early as 1830 by De Jussieu[1] and its taxonomic position is as follows:-

Kingdom: plantae
Division: Magnoliophyte
Class: Magnoliopsida
Order: sapindales
Family: Maliaceae
Genus: Azadirachta
Species*: A.indica*
Scientific name: Azadirachta *indica*

Neem which belongs to the family Meliaceae, originated from South Asia, but grows widely in India, Pakistan and other tropical and sub-tropical parts of the world (Bokhari and Aslam, 1985; Von Maydell, 1986). The tree was introduced in Nigeria from Ghana, and it was first grown from the seeds in Maiduguri, in the then Bornu Province (now Borno State), Nigeria, in 1928 (National Research Council, 1992; Nwoeabia, 1994). The Neem tree is significant in Nigerian forestry because it constitutes the largest population of trees, especially in the Northern States. It was nicknamed 'Dogon Yaro' after the first caretaker of the Neem tree Nursery in Maiduguri. Neem is a moderate sized to large, usually evergreen tree, with a fairly dense crown and glabrous leaves divided into leaflets. The bark is fairly thick, furrowed longitudinally or obliquely and is dark grey outside and reddish brown inside. The tree in Maiduguri flower throughout the year but fruits during the cold harmattan season which corresponds with the winter of temperate climates. The

fruits are yellowish green when ripe and have a sweetish pulp containing one seed. In Northern Nigeria, the neem plant is used in traditional circles for the treatment of general body pain after child delivery, pyorrhea, and intestinal worms (Bokhari and Aslam, 1985). Based on this traditional and other uses of *Azadirachta indica*, this study was conducted to ascertain its potentially pharmacologically active components

MATERIALS AND METHODS

Collection of Samples: *Azadirachta indica* plant leaves were collected from the college premises. Then ensure that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves thoroughly and a particular amount of leaves dried under shade and hand crushed to obtain a 138.1 g.

Extraction of plant material: Extraction was carried out in 500ml Ethanol at 60^{o}C for 8 hours using the soxhlet extractor. Extract was then concentrated and stored at room temperature (20^{o}C).

Phytochemical screening: The phytochemical analysis of the extract of *Azadirachta indica* leaves for alkaloids, saponin, tannins, proteins, Cardiac glycoside, Phenols, steroids and flavonoids was carried out. The powdered leaf was extracted with the required solvent and necessary reagent added to the right quantity of the extract. All observations were recorded.

Qualitative estimation: Qualitative analysis was done to identify the presence of the following phytoconstituents; alkaloids, flavonoids, tannins and phenols, steroids and terpenoids, saponins, Carbohydrates, glycosides, proteins and amino acids using standard procedures.

Test for Alkaloids: (a).Dragondroff test: plant extract add methanol(10 ml) then filter 2 ml filtrate add 1% HCL (0.5 ml conc. HCL +49.5ml distilled water) + steam 1 ml filtrates add 6 drops of dragondroff reagent. orange ppt observed and it represent presence of alkaloid.

Preperation of Dragondroff's reagent: -- 0.5 gram of bismuth nitrate in to an empty beaker. Add 10 ml of concentrate HCL add Pour 4 gram of potassium iodide (into another beaker). Add a little water and stir until KI is completely dissolved Mix two solution dark orange colour obtained

Test for Flavonoid:

(a) Alkaline reagent test: Extract was treated with 10% NaOH solution; formation of intense yellow colour indicates presence of Flavonoids.

Test for tannins: 200mg 2 plant material+10 ml distilled water and then filter take 2 ml filtrate add 2ml $Fecl_3$ (neutral) Blue black ppt or dark green ppt indicate the presence of tannins and phenol.

Test for saponins: 200 mg plant extract and 5 ml distilled water frothing persistence indicate presence of saponin.

Test for cardiac glycosides: (Killer Killani test): 2ml filterate add 1ml glacial acetic acid add $Fecl_3$ and conc. H_2SO_4. Green blue ppt indicates the presence of glycosides.

Test for steroids: 200 mg plant material add 10 ml chloroform then filtrate add 2ml acetyl chloride add conc. H_2SO_4 Bluish green ring indicate presence of steroids

Phenolic test: 500 mg extract dissolved in 5ml of dist. Water add few drops of neutral 5% $Fecl_3$. solution. Dark green colour indicate the presence of phenolic compound

RESULTS

Table 1: Phytochemical components of Neem leaf ethanol extract

Sr. No.	Components	Components	Components
1	Alkaloids	Dragendorff's	+
2	Saponins	Frothing	+++
3	Tannins	Ferric chloride	++
4	Glycosides Combined	Killer killani test	++
5	Flavonoids	Yellow colour	+
6	Tannins	Blue green ring	+

Quantitative estimation of phytochemical constituents of Neem:-

Quantitative test for flavonoids: 2 gram of the plant sample extracted with 20 ml of 80% aqueous methanol at room temp. The whole solution was filtrate through whatmann filter paper number 125mm.The filtrate was later transferred into a crucible and evaporate into dryness over a water bath and weighted to a constant weight.

Test for tannin: In this method 500 mg of crude extract was shaking with 50 ml dist. water. For an hour and filtrate was then subjected to. 1M $Fecl_3$ in. 1N HCL and potassium ferrocynide 0.008M. After which the absorbance within in 10 min 420 nm the conc. of tannins was expressed as tannic acid equivalent in mg/g (TAEMG/G).SS

CONCLUSION

The phytochemical test results indicated high scores for saponins, moderate scores for tannins and glycosides while alkaloids, terpenes and flavonoids had low scores. Azadirachtin extracts from the leaves of the Neem tree has been reported to have strong biological activities against insect pests, but with very low toxicity to mammals and the environment. Therefore, the wide use of the neem plant is attributable to the presence of these bioactive compounds, which may explain its many traditional uses against various ailments. Further research is recommended on this as a confirmation.

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