



Research Article

Phytochemical screening and antimicrobial activity evaluation of aqueous and ethanolic extracts of the leaf of *Azadirachta indica* Juss (neem) on some microorganisms

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Azadirachta indica Juss (neem) is a plant which has been used for a long time as traditional medicine for household remedy against various human ailments from antiquity. To evaluate the scientific basis for the use of *Azadirachta indica*, both aqueous and ethanolic extracts of the dried leaves of the plant were subjected to phytochemical screening and determination of anti-microbial activity on six different species of bacteria and a fungus. The phytochemical screening of the aqueous and ethanolic extracts of dried powdered leaves of the plant was done using standard methods. The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against the microorganisms using agar well diffusion method. The Phytochemical screening of the test plant revealed the presence of saponins, alkaloids, cardiac glucosides, phenols, resins, tannins, terpenes and steroids. Although, both plant extracts had antimicrobial effects against the test organisms, the aqueous extracts were found to show greater anti-microbial effect than ethanolic extract. Thus, the mean diameter zones of inhibition ranged from 0.03mm-40.00mm for aqueous extract and 0.50mm-21.00mm for ethanolic extract at the highest concentration of 50mg/ml. The finding of this study supports the use of neem leaf in the treatment of various microbial infections by alternative systems of medicine.

Keywords: Phytochemical, Antimicrobial, *Azadirachta indica*, Aqueous and Ethanolic Extracts.

INTRODUCTION

Herbal medicines have been known to man for centuries. Practitioners of traditional medicine (PTM) have described therapeutic efficacy of many indigenous plants for several disorders. Neem plants (*Azadirachta indica*) are mostly trees and rarely shrubs that belong to family Maliacea (Margaret, 1965). The plant has been used for a long time in agriculture and medicine (Natarajan *et al.*, 2003). Neem is a widely distributed Indian indigenous plant. In India the tree is regarded as a "village dispensary" (Kirtikar and Basu, 1975). People from different countries have also learnt about this miraculous plant, carried seeds with them to grow in their respective countries. Indians settled abroad too followed the suit and soon neem started growing in many countries (Micheal, 2002). Neem is also found in Nigeria (Bodurin, 1999). The importance of the neem

tree has been recognized by US National Academy of Sciences, which published a report in 1992 entitled "Neem-a tree for solving global problems" (Biswas *et al.*, 2002). It is established that many scientific studies that neem seeds contain chemical compounds to control more than 100 species of insects and microorganisms (Vaideki *et al.*, 2007). Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity (Chopra *et al.*, 1958; Thakur *et al.*, 1981). The excellent bioactivity of this neem products are attributed to the chemical compounds such as nimbin, nimbidin and salanin (Rao *et al.*, 1986). Neem leaves are reported as the main sources of the active compounds obtained from the plant. Neem leaf contains several valuable components such as

isoprenoids that include terpenoids containing limonoids, azadirone and its derivatives (Xu *et al.*, 2010).

The medicinal properties of the plant were studied by several workers. The antipyretic effect (Kirtikar and Basu, 1975; Okpanyi and Ezekwu, 1981) antimalaria effect (Tella, 1977; Rochankij *et al.*, 1985) antidiabetic effect (Shukla and Bhandari, 1973), antifertility effect (Sinha *et al.*, 1984), effect on the central nervous system (Phillai and Shanthakumari, 1984), cardiovascular effect (Thompson and Anderson, 1978) and wound healing (Jayaprakasan *et al.*, 2014) were some of the studies of earlier workers. *A. indica* has been shown to possess anti-microbial properties by several studies. Rao *et al.* (1986) reported the anti-microbial activity of the seed oil against a variety of pathogens. Oils from the leaves, seeds and bark possess antibacterial action against certain bacteria (Khan and Wassilar, 1987). Extracts of neem leaf, neem oil and seed kernels are effective against certain human fungi (Biswas *et al.*, 2002). More than 135 compounds have been isolated from different parts of neem and several reviews have also been published on the chemistry and structural diversity of these compounds (Fujiwara *et al.*, 1982; Khan and Wassilar, 1987). In view of the reported medical properties of the plant this work is intended to determine the chemical constituents of the leaf by carrying out its phytochemical screening and to investigate the antimicrobial activity of the aqueous and ethanolic extracts of the leaf of *Azadirachta indica* on some microorganisms.

MATERIALS AND METHODS

Sample Collection and Preparation of Plant Materials

Fresh leaves of *A. indica* Juss were collected from Jos North Local Government area, Plateau State, Nigeria and authenticated at the Botany Department of University of Jos. After collection, the leaves were dried in the shade. The dried leaves were then ground to powder using an electric blender and kept in the refrigerator prior to use. The aqueous and ethanolic extracts of the plants were extracted from 100g of dried powdered leaves with 400ml sterile distilled water and 400ml ethanol respectively using Soxhlet method (Nwachukwu *et al.*, 2006). The extracts of the plant were evaporated in an oven at 40°C, and then used for Phytochemical screening and microbiological studies (Reeves *et al.*, 1978).

Phytochemical Screening of Aqueous and Alcoholic Extracts of *A. indica* leaf

The phytochemical screening for major constituents was undertaken using standard qualitative methods as described by Trease and Evans (1989), Harbone (1985), Odebiyi and Sofowora (1990) and Sofowora (1993). The phytochemical screening of the aqueous and alcoholic extracts of the leaf of *A. indica* was

carried out for the presence alkaloids, cardiac glucosides, flavonoids phenols, resins, saponins, tannins, terpenes and steroids using standard phytochemical methods. The phytochemical screening of the ethanolic extracts of the plant was carried out in order to elucidate the chemical constituents (bioactive agents) responsible for their antimicrobial and therapeutic activities.

Tests for Alkaloid

Preliminary test (Dragendorff reagent test)

A few drops of Dragendorff reagent were added to 2.0ml of the extract and a solution of potassium bismuth iodine was also added and observed for orange colouration.

Confirmatory test (Wagner reagent test)

A few drops of the Wagner Reagent were added to 2.0ml of the extract and a solution of iodine in potassium iodine was also added and the formation of deep brown precipitate would indicate the presence of alkaloid (Trease and Evans, 1989).

Tests for Cardiac Glycoside

Preliminary test (Lieberman's test)

Two millilitres of the plant extract was added to 2.0ml of acetic anhydride and cooled in ice. Sulphuric acid was added carefully along the side of the test tube. A colour change from violet to blue green indicates the presence of steroid nucleus (i.e. a glycone portion of the cardiac glycoside).

Confirmatory test (Salkowski test)

Two millilitres of the extract was dissolved in 2.0ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase indicates the presence of cardiac glycoside (Sofowora, 1993).

Test for Flavonoids

A few drops 10% of lead acetate solution was added to 2.0ml of the extract in a test tube. The observation of either cream or light yellow colourations confirms the presence of flavonoids (Harbone, 1985).

Test for Phenols

Two millilitres of the extract was added to 2ml of ferric chloride. A deep blush green solution indicates the presence of phenols (Odebiyi and Sofowora, 1990).

Test 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,

Table 1. Phytochemical Screening of Aqueous and Ethanolic Leaf Extracts of *Azadirachta indica*

Chemical Component	Aqueous Extract	Ethanol Extract
Alkaloids	+	+
Cardiac glycosides	+	+
Flavonoids	+	+
Phenols	+	+
Resins	+	+
Saponins	+	+
Tannins	+	+
Terpens and Steroids	+	+

+ = Presence of chemical component.

rapidly changing to violet indicates the presence of resins (Trease and Evans, 1989).

Test for saponins

Five millilitres of distilled water was added to 2.0ml of the extract in a test tube. This was shaken vigorously after which a few drops of olive oil was added. Formation of an emulsion will indicate the presence of saponins. Also the formation of persistent foams during plant extraction or during the concentration of plant extract is reliable evidence that saponins are present (Sofowora, 1993).

Tests for Tannins

Preliminary test

One millilitre of the extract was diluted with 4.0ml of distilled water (in a ratio 1:4) and a few drops of 10% very dilute ferric chloride solution was gradually added to the aqueous extract. The presence of blue or green precipitate or colourations shows the presence of tannins.

Confirmatory test

A few drops of lead acetate solution were added to 2.0ml of the extract. The resulting solution was observed for brown precipitate which indicates the presence of tannins (Sofowora, 1993).

Test for Terpenes and Steroids

One millilitre of acetic anhydride was added to 2.0ml of the extract and then concentrated sulphuric acid was carefully added down the side of the test tube. An observation for reddish brown colour at the interphase indicates the presence of terpenes and steroids (Harbone, 1973).

Source and Preparation of Microorganisms

The microorganisms used in this study were obtained from Microbiology Unit of Jos University Teaching Hospital (JUTH), Nigeria. The microorganisms used for the investigation included *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella* sp,

Pseudomonas aeruginosa, *Salmonella typhi* and *Candida albicans*. The cultures of the microorganisms were on agar slopes at 4°C and were sub-cultured into nutrient broth and incubated at 37°C for 24 hour prior to sensitivity testing in order to obtain a more vigorous population.

Sensitivity Testing

Before carrying out the antimicrobial test, the ethanolic and aqueous plant extracts and the standard were prepared using doubling dilution method described by (Taura and Oyeyi, 2009) to obtain 50mg/ml, 25mg/ml and 12.5mg/ml. The antimicrobial activity of the plant extracts was determined using agar well diffusion method (Thornberry, 1983; Irobi *et al.*, 1996; Akande and Hayashi, 1998). About 0.5ml of the standardized portion of the new microbial culture was aseptically transferred into Petri dishes containing Nutrient Agar (NA) for bacterial isolates and Potato dextrose Agar (PDA) for *Candida albicans* and left for about 20 minutes to allow the microorganisms fix on the media. Wells where extracts were to be introduced into the plates were carefully marked using sterile cork borer (6mm diameter) and small drops of extract of various concentrations (50mg/ml, 25mg/ml and 12.5mg/ml) were added into the wells. A well was also made at the central portion of the agar medium and drops of sterile distilled water and or 95% ethanol were placed therein to serve as controls. The plates which were prepared in triplicates were incubated at 37°C and the zones of inhibition were measured after 24 hours (Mudi and Ibrahim, 2008). The presence of zones of inhibition was regarded as the evidence of antimicrobial action. The zones of inhibition were measured with a ruler at right angles across the zones to find the average diameter in millimeters.

RESULTS AND DISCUSSION

Preliminary phytochemical investigation of aqueous and ethanolic leaf extracts of *Azadirachta indica* are presented in Table 1. The result revealed the presence of alkaloids, cardiac glucosides, flavonoids, phenols, resins, saponins, tannins, terpenes and steroids in both aqueous and ethanolic leaf extracts.

The results obtained from the present study showed

Table 2. Anti-microbial Effects of different Concentrations of Aqueous and Ethanolic Leaf Extracts of *Azadirachta indica* on Some Microorganisms

Organism	Mean diameter of zone of inhibition (mm) at Different concentrations (mg/ml)					
	AE			EE		
	50.0	25.0	12.5	50.0	25.0	12.5
<i>Staphylococcus aureus</i>	40.00	35.00	-	0.50	0.35	-
<i>Bacillus subtilis</i>	21.00	19.00	-	21.00	18.00	-
<i>Escherichia coli</i>	28.00	25.20	-	1.00	0.60	-
<i>Klebsiella sp</i>	0.04	0.30	-	4.00	2.00	-
<i>Salmonella typhi</i>	26.00	20.50	-	3.00	2.20	-
<i>Pseudomonas aeruginosa</i>	0.03	0.20	-	4.00	3.00	-
<i>Candida albicans</i>	12.30	10.00	-	18.00	5.00	-

AE = Aqueous Extract
 EE = Ethanolic Extract
 - = No inhibition

that the aqueous and ethanolic extract of the leaf of *A. indica* have some antimicrobial activities against the test microorganisms (Table 2). The aqueous and alcoholic extracts of the leaf of *A. indica* showed anti-microbial activity against all the test organisms at concentrations 25mg/ml and 50mg/ml; however both extracts showed no activity at concentration 12.5mg/ml. On comparing the two extracts, aqueous extract seem to exhibit higher anti-microbial effect than the ethanolic extract except with *Bacillus subtilis*, which showed almost similar sensitivity to both extracts. This may be suggesting that the anti-microbial activity of *A. indica* leaf extract seemed to depend on its polar constituents with the aqueous extract being more polar than the ethanolic extract (Onoruvwe, 1965).

The degree of inhibition by aqueous extract was observed to be highest in *S. aureus* and lowest in *Pseudomonas aeruginosa*. Results on the effect of the ethanolic extract of the plant showed that the growth of *B. subtilis* was most strongly inhibited followed by *Candida albicans* at 50mg/ml, while activity against *S. aureus* was least at the same concentration. The crude extract of the leaf of *A. indica* was active against Gram positive and Gram negative bacteria as revealed in the study. This finding confirms the studies of Rao *et al.* (1986), which reported that *A. indica* possesses a wide spectrum of antibacterial activities. Also results obtained show that the extracts of neem plant inhibited the growth of *Candida albicans* as corroborated by the work of Khan and Wassilew (1987).

The inhibition of various microbial isolates used in this study by the leaf of *A. indica* can be extrapolated to explain that it could be effective in the treatment of infection caused by the organisms (George, 1980; Burkill, 1985). *Staphylococcus aureus* is found in wounds and also causes skin infections; *Bacillus subtilis* is involved in gastroenteritis, *Salmonella typhi* causes typhoid fever, *E. coli* is a common organism involved in diarrhoea of bacterial origin, while *Candida albicans* has been implicated in thrush.

The anti-microbial property exhibited by the leaf extracts of *A. indica* may be due to the presence of individual bioactive ingredients in the plant. These chemical constituents are known to possess anti-microbial properties (Erique, 1988). The presence of glycosides in the leaf of *A. indica* is important in therapeutic use of this plant because of their ability to

increase the force of systolic concentration (Tyler *et al.*, 1988). Phenolic compounds extracted from many plant parts have shown an excellent antioxidant capacity (Harbone, 1985). Since the extracts showed no activity at concentration 12.5mg/ml, it is possible that the quantity of the active ingredients present at this concentration is not sufficient to encourage their inhibition of the microorganisms. The high inhibitory effect exhibited by neem leaf extracted on some of the test microorganisms further supports the traditional uses of the plant for curing various infections and thus confirming its therapeutic potency.

The observed activity of the leaf extracts of *A. indica* against all the test microorganisms may present it as a useful agent in treating bacterial and fungal-based infections. Neem leaf can serve as a potential source of drugs which may be a subject of great interest among drug and cosmetic manufacturers.

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