

# Phytochemical Extraction and Antimicrobial Studies on Crude Leaf Extract of *Azadirachta indica* (Neem) in Semi-Arid Region of Borno State, Nigeria

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## ABSTRACT

Crude leaf extracts of *Azadirachta indica* (Neem) was subjected to in vitro phytochemical screening and antimicrobial assays against some selected microbial pathogens by well diffusion technique. A qualitative preliminary phytochemical screening of crude leaf extracts showed the existence of bioactive phytocomponents such as alkaloids, flavonoids, phenols, tannins, and reducing sugars in the leaves. Soxhlet extraction was also performed by using different solvent system for the analysis, chloroform, hexane, methanol, and ethyl acetate. Extracts of leaf showed significant antimicrobial activity against microbial pathogens. Chloroform and methanol extracts showed highest and moderate activity against the tested microbial pathogens, while other extracts showed lowest activity. The bioactive compounds crude leaf extracts of neem subjected to antimicrobial activity against pathogens shows remarkable sensitivity with higher zone of inhibition ranging from 20mm to 27mm against streptococcus mutans, E. coli, streptococcus pyogenes using chloroform, ethyl acetate and Hexane extract. While moderate zone of inhibition ranging from 14mm to 17mm was obtained against staphylococcus aureus, pseudomonas aeruginosa, Candida albicans using methanol, chloroform. Whereas weak zone of inhibition ranging from 10mm to 12mm was obtained against Aspergillus niger, Aspergillus flavus and streptococcus pyogenes using methanol, hexane, chloroform and ethyl acetate. The bioactive phytocomponents of those showing antimicrobial activities could be exploited as the source of a potent bioactive ingredient for ethnobotanical approach and to build up potential pharmaceutical products to improve the quality of health care in the study area.

**Keywords:** Phytochemical Extraction crude leaf extract, antimicrobial studies, neem, *Azadirachta indica*.

## INTRODUCTION

*Azadirachta indica* (Neem) is a subtropical tree native to the drier regions of Asia and Africa (Omprakash, 2014; Ahmad

et al 1998). Neem (*Azadirachta indica* Juss) is well known for centuries in India and Africa as insecticides, fungicides, anticonceptionals in popular medicine

almost every part of this tree seeds, leaves, roots, bark, trunk and branches has multiple uses (Jingfa et al; 2010; Chaturvedi et al; 2003).

According to (Susmitha et al; De Jussieu (1830) the neem tree has been described as *A. indica* as early as 1830 and its taxonomic position is as follows:

Order	-	Rutales
Suborder	-	Rutales
Family	-	Meliaceae
(Mahogany family)		
Subfamily	-	Melioideae
Tribe	-	Meliceae
Genus	-	<i>Azadirachta</i>
Species	-	<i>Indica</i>

There are biologically active compounds in meliaceae species (*Azadirachta indica* and *Melia azadirachta*) which are called limonoids, other bioactive compounds include Nimbidin, Nimbin which are anti-inflammatory, anti-bacterial, and anti-fungal respectively (Siddiqui and Ali, 1997). The Neem seed has also been used as traditional medicine to treat infections and could be used for controlling airborne bacterial contamination in residential vicinity (Saseed, et al., 2008, Shrivastava and Swarnkar 2014). According to (Sonia Bajaj and Sarinivasan, B.P. 1999) Aqueous extract of neem leaf has good therapeutic potentials as anti-hyperglycemic and anti-inflammatory agents. Hassan, et al., 2010 suggests that the aqueous extract of neem has a powerful chemotherapeutic and viral agent.

In recent years neem bioactive compounds secondary plant metabolites and phytochemical with known pharmacological activities have been widely investigated as a source of medicinal agents (Krishnaraju et al., 2006.; Sowjanya et al., 2013). Thus it is expected that phytochemical extraction with adequate antimicrobial efficiency will be used for the treatment of many microbial infections (Balandrin et al., 1985).

Several phytochemical assays have been reported, the major bioactive compounds were explored via phytochemical

surveys are alkaloids, steroids, triterpenoids and essential oils (Lozoya and Lozoya 1989, Sowjanya et al., 2013). Phytochemical of crude leaf extract of neem plant was carried out according to the methods and described by Dey and Sitaramants 1957., Sowjanya 2013). The purpose of the present study was to explore the antimicrobial activity of crude leaf extract of neem (*Azadirachta indica*) and determine its potential as a source of bioactive compounds for chemotherapeutic studies.

## **MATERIALS AND METHODS**

### **Collection of sample**

The crude leaves of neem (*Azadirachta indica*) were collected from the botanical garden of University of Maiduguri. The plant was identified accordingly to various literatures and with the help of taxonomic key in the library.

### **Preparation of crude leaf extract**

The collected leaves were chopped into small pieces, completely dried and coarsely powdered with suitable homogenizer. The powdered extract was obtained after homogenization, subsequently, allowed for successive extraction with organic solvents such as methanol, chloroform, ethyl acetate, and hexane by using Soxhlet extraction method subsequently, the extracts were collected and distilled off on a water bath at atmospheric pressure and emptied any trace solvent in the vacuum. The extracts obtained were subjected to phytochemical assay and antimicrobial studies.

### **Microorganisms**

Microorganisms were obtained from Department of Veterinary Microbiology Laboratory, University of Maiduguri. Microorganisms were maintained at 40°C on nutrient agar slants. Subsequently, they were subcultured and reconfirmed by gram staining technique.

### **Phytochemical Analysis**

Qualitative Phytochemical analysis of the crude powder of the neem leaf were screened for the presence or absence of secondary metabolites such as flavonoids,

steroidal compounds, phenolic compounds, tannins and saponins using standard procedure as described by Harborne 1998.

#### **Phytochemical assay of crude leaf extract of neem (Azadirachta indica)**

Phytochemical investigations were carried out for all the crude leaf extracts using aseptic and standard techniques.

1. Detection of alkaloids from crude leaf extracts of neem (*Azadirachta indica*): The extract was dissolved in dilute hydrochloric acid and solution was clarified by filtration. Subsequently, Mayer's test was conducted by treating filtrate with Mayer's reagent (potassium mercuric iodide).

The presence of alkaloids was determined by formation of a yellow precipitate. Furthermore, Wagner's test was carried by addition of Wagner's reagent (iodine in potassium iodide) on the filtrate. Presence of alkaloids was determined by formation of brown/reddish precipitate (Siddiqui and Ali, 1997; Sonjanya et al, 2013). Other phytochemical examination include Dragondroff Test for the determination of alkaloids when filtrate was treated with solution of potassium Bismuth iodide to observe formation of red precipitate indicating the presence of alkanoids.

2. Detection of phenols from crude leaf extracts of neem (*Azadirachta indica*): The detection of phenols was carried out by ferric chloride test. This test entails addition of three drops of freshly prepared 1% ferric chloride and potassium ferrocyanide. The formation of bluish-green colour indicates as positive. While the methanol extract was dissolved in water, few crystal of ferric sulphate was added to the mixture. Formation of dark-violet color indicated the presence of phenolic compounds.
3. Detection of flavonoids from crude leaf extract of neem: Flavonoids were investigated using alkaline reagent test. The extract was treated with new drops of sodium hydroxide solution. The

formation of intense yellow color, which becomes colorless on addition of dilute HCl acid, indicates the presence of flavonoids. And lead acetate test were used for further confirmation test. The extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids (Siddiqui and Ali, 1997).

4. Detection of Reducing Sugars :

The crude leaf extract were dissolved in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Fehling's solution was added on the filtrates (hydrolyzed with dil. HCl neutralized with alkali and heated with feehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

5. Detection of Tannins

The determination of tannins in the crude leaf extract of neem was carried out using ferric chloride test. The extract was dissolved in water. The solution was clarified by filtration; 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in color to bluish black (Lyengar, 1995).

6. Detection of fatty acids:

The extract was mixed with 5 ml of ether and allow for evaporation on filter paper to dry. The appearance of transparence on the filter paper indicates the presence of fatty acids (Siddiqui and Ali, 1997).

#### **Antimicrobial activity assay**

Antimicrobial activity of the crude leaf extract of neem was determined by Agar well-diffusion technique on nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) Plates. The extracts were swabbed (sterile cotton swabs) with 24 hours and 48hours broth culture of bacteria and fungi respectively (Anon, 1996; Perez et al, 1990). Wells are made in nutrient agar plate using cork borer (5mm diameter). 0.5ml of 65mg/ml concentrations of plant extracts were added using sterilized dropping

pipettes into the wells and left to diffuse at room temperature for few hours. The plates were incubated at 37<sup>0</sup>C for 24 hours for bacterial and 26-28<sup>0</sup>C for fungal pathogen. The same volume of extraction solvent for control for leaf extract was also maintained and after overnight incubation the plates were observed for the zone of inhibition (ZI) and the diameter of the inhibition zone were measured and compared with standard values (Kirby Bauer et al, 1966).

## RESULT

Phytochemical Assay of crude leaf extract of neem (*Azadirachta indica*):

The phytochemical assay was performed for the detection of alkaloids, flavonoids, phenols, tannins, reducing sugars and fatty acids. A qualitative phytochemical analysis revealed that, alkaloids are present highly in methanol moderately in chloroform and water extracts, flavonoids are present moderately in ethyl acetate and water extracts, absent in other extracts. While phenolics are faintly present in chloroform moderately methanol and ethyl acetate extracts. Tannins are faintly present in ethyl acetate and water extracts. Fatty acids were completely absent in all the extracts.

### Antimicrobial Bioassay

The antimicrobial activities of crude leaf extract of neem were determined by the Kirby-Bauer Agar diffusion method (1966). The active phytochemicals were analyzed and further antimicrobial activity of the neem extracts was assayed in vitro under aseptic conditions in the biosafety chamber. The diameter of zone of inhibition as indicated by clear area which was devoid of growth of microbes was measured and recorded (Cowan, 1999). The results of antimicrobial activity of methanol, hexane, chloroform and ethyl acetate crude leaf extracts of neem against bacterial and fungal pathogens were tabulated in the table 2. It revealed that chloroform, methanol leaf extract exhibited significant activity compared to the other extracts. The chloroform leaf extracts showed the highest

activity against staphylococcus aureus and streptococcus mutans. While methanol extracts showed maximum activity against staphylococcus aureus streptococcus mutans respectively. The ethyl acetate and hexane extracts showed moderate activity against some bacterial and fungal pathogens.

## DISCUSSION

The beneficial phytochemicals of plant materials typically result from secondary metabolites present in the plant. A significant number of studies have been carried out for screening of phytochemicals for ethno botanical and pharmacological study. In this research, the bioactive phytochemicals of crude leaf extracts of neem were assayed in vitro by agar well diffusion method against eight microorganisms (5 bacterial and 3 fungal species). Table 2 shows the antimicrobial activity of methanol chloroform, ethyl acetate and hexane extracts of the crude neem plant. Chloroform and methanol extracts showed the presence of alkaloids highly in methanol, moderately in chloroform and Water extracts. Flavonoids are present moderately in ethyl acetate extracts, absent in other extracts. The phytochemical analysis of *Azadirachta indica* extract has earlier been reported by Kubmarawa, et al., 2008; Kraus et al., 1981). Bui et al (2009), also observed the presence of anti-nutrients like alkaloids, flavonoids in the aqueous extract of the leaves of *Azadirachta indica* (neem). According to Benneth and Wallsgrave (1994), Osbourn (1996) a large number of phytochemicals have been reported to have anti-fungal activity. The antimicrobial activities of the crude leaf extract of neem shows remarkable sensitivity against bacterial and fungal isolates use in this research as shown in table 3. Higher zones of inhibition ranging from 20mm to 27mm was recorded against streptococcus mutans, E.coli, S. pyogenes, using chloroform, ethyl acetate and Hexane extract. While moderate zone of inhibition ranging from 14mm to 17mm was recorded against staphylococcus

aureus, pseudomonas aeruginosa, Candida albican using methanol, chloroform and weak zone of inhibition ranging from 10mm to 12mm was recorded against A. niger, A. flavus, streptococcus pyogenes using methanol, Hexane, chloroform and ethyl acetate. The antimicrobial activity of many plant extracts had been reported and classified as strong, medium or weak by Zaika 1998 and Susmitha et al., 2013. This observation suggests that, the inhibition produced by the phytochemicals crude leaf extract of neem against bacterial and fungal pathogens depends upon various extrinsic and intrinsic parameters. This is also attributed to variable diffusability in agar medium. Thus the antimicrobial properties of the crude leaf extract of neem may be due to the availability of phytochemicals (Alkaloids, flavonoids

sugars) which promote antimicrobial activity (Akujobi et al., 2004., Ogbulie et al., 2007.; Mohammed et al., 2012). Hence, these findings provided a preliminary scientific formation on the potential of crude leaf extract of neem to control microbial pathogens. The observed antimicrobial and phytochemical properties of the phytochemicals of crude leaf extract of neem tested against different microbial pathogens confirmed to have a potential antimicrobial property. The findings of this research also collaborated its use in the traditional medicine in the study area. Hence, further pharmacological evaluation should be conducted so that the phytochemicals of crude leaf extract of neem could be exploited as the source of potent bioactive ingredients to improve the quality of health care in the study area.

**Table 1: Phytochemical assay of Neem (Azadirachta indica)**

Chemical constituent	chloroform	methanol	Hexane	Ethyl acetate	Acetone	Water
Alkaloids	**	***	-	-	-	**
Flavonoids	-	-	-	**	-	**
Phenol	*	**	-	**	-	-
Reducing Sugar	*	-	-	*	*	-
Tannins	-	-	-	*	-	*
Fatty acids	-	-	-	-	-	-

Key → Absent, \* = faintly present, \*\* - moderately present, \*\*\* - highly present.

**Table 2: Antimicrobial Activity of crude leaf extract of neem (Azadirachta indica).**

Test organism	Methanol	Chloroform	Hexane	Ethyl acetate
P. aeruginosa	14	14	6	10
Staph. aureus	22	27	10	12
S. pyogenes	12	12	8	10
E.coli	12	14	8	12
Strept. mutans	20	27	10	12
Cand. albican	14	10	8	12
A. Niger	10	10	6	8
A. flavus	12	12	10	6

**Table 3: Zones of inhibition of the crude leaf extract of neem on Bacterial and fungal pathogens**

Test organism	Methanol	Chloroform	Hexane	Ethyl acetate	Control (AMP)
P. aeruginosa	12	16	10	14	28
Staph. aureus	14	14	10	12	26
S. pyogenes	8	14	22	10	24
E.coli	14	16	12	27	23
Strept. mutans	12	27	14	10	26
Candida albicans	12	10	12	14	10
A. Niger	12	10	10	12	11
A. flavus	12	11	12	10	12

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