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Phytochemical Screening and Antimicrobial Activities of Extracts of Acacia nilotica Against Selected Pathogens

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Abstract
Traditional medicine systems plants are the major sources of substances used in managing and/or treating many diseases conditions. This study was aimed at screening the phytochemicals present in the seed and pod of *Acacia nilotica* using different solvents (n-hexane and methanol) and to determine the anti-oxidant and anti-microbial activity of the plants extracts using some selected pathogens. Qualitative phytochemical screening revealed the presence of alkaloid, flavonoids, glycosides and tannins in both extract of *Acacia nilotica* seed while flavonoids was however only present in methanol extract, the results further revealed that Saponins was absent in all the seed extracts. For the pod extract, tannins, alkaloids, saponins and glycosides were present while alkaloid was absent in all the extracts. In vitro antioxidant studies shows both seed and pod extracts to possess strong antioxidant activity as they compare favorably with standard antioxidant (Ascorbic Acid). Anti-microbial studies shows the extract to be very effective against *S. aureus P. aeruginosa, S. pyogenes, S. typhi* and *C. albica* pathogens. Thus this study demonstrated *Acacia nilotica* seed and pods to possess strong anti-oxidant and anti-microbial properties which might be as a result of presence of some bioactive compounds.
Keywords: *Acacia nilotica;* Anti-oxidant; Anti-microbial; Phytochemicals; Pods and Seeds.
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1.0 Introduction

Traditional medicine commonly known as ethno-medicine or folk medicine, is the oldest form of health care system and is used in the prevention and treatment of various diseases. In the traditional medicine systems plants are the major sources of substances used in the management and/or treatment of many diseases. The developing countries are leading in the patronage of traditional medicine, Nigeria inclusive (Duraipandiyan *et al*., 2006).

According to WHO, 80% of rural communities rely on plants for their health care. This has created growing interests in research on plants and plant derived compounds called phytochemicals. These compound are thought to be responsible for many bioactive and pharmacological activities. In depth studies of these compound could improve our understanding of how traditional medicine men used plants in providing health care delivery.

The tradition of using plant products to treat a number of diseases starts with the beginning of human civilization. The earliest document shedding light on the use of medicinal plants is Hindu Culture, written between 4500 and 1600 BC (Restogi and Mehrotra, 2002). The use of traditional medicine or natural products is old as human civilization (De pasquale, 1984). Conventional drugs were reported to have some health-related side effects, microbial resistance and inefficiency. Man usually tend to search for natural medication from plant with less or no side effect, safe and effective with many biological activities such as analgesic, antimicrobial, wound-healing, antioxidant, anticancer, antidiarrheal activities (Sasidharan *et al*, 2010).

Acacia nilotica plant is found throughout Borno and Yobe states and it is traditionally used for the treatment/management of many human disease conditions by these communities. A brief survey of the traditional medicinal usage of the plant shows that it is used to treat cancer, ulcer, diabetes and infections. Although a number of studies have reported different bioactive roles or pharmacological effects to these plants, there's the need to carry out well coordinated study to identify which part of the plant contains what phytochemicals and what medicinal effect it provides. These detailed studies would reveal the compounds responsible for the acclaimed medicinal benefits for which the plants are used by the traditional system. Furthermore, such scientific probes can lead to the identification and development of potential novel drugs/agents for use by both orthodox and traditional medicine as observed by Principe (1985), Cowan (1999) and Buttler (2005).

Following the rebellion in the "golden era", while about all groups of vital antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered and now a days and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance. For such motive, discovery of new antibiotics is an utterly vital objective. Nowadays natural products remain one of the key sources of new drug molecules. Plants and other natural sources can provide a huge range of complex and

structurally diverse compounds. Recently, researchers are being focusing on investigating plant and essential oils, microbial extracts, pure secondary metabolites as budding antimicrobial agents (Balouirin *et al*., 2016).

Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, the pathologies caused by diabetes, rheumatoid arthritis, and neuro degeneration in motor neuron diseases (Nunomura *et al*., 2006). In many of these cases, it is unclear if oxidants trigger the disease, or if they are produced as a secondary consequence of the disease and from general tissue damage; one case in which this link is particularly well-understood is the role of oxidative stress in cardiovascular disease. Here, low density lipoprotein (LDL) oxidation appears to trigger the process of atherogenesis, which results in atherosclerosis, and finally cardiovascular disease (Van Gaal *et al*., 2006). Oxidative damage in DNA can cause cancer. Several antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase protect DNA from oxidative stress. It has been proposed that polymorphisms in these enzymes are associated with DNA damage and subsequently the individual's risk of cancer susceptibility (Abner *et al*., 2011).

This study is aimed at providing scientific explanation for the continued used of *Acacia nilotica* as anti-microbial and anti-oxidant agents in the treatment of various diseases.

2.0 Materials and Methods

2.1 Collection and identification of plant materials

The plants material (Pod and Seed) of *Acacia nilotica* were collected at Auno Local Gorvernment Area of Maiduguri, Borno state and identified by a plant taxonomist from the Dept. of Biological Sciences, University of Maiduguri. The plant materials were cleaned, dried under shade at ambient laboratory temperature and powdered by using local mortar and pestle into coarse powder and then kept in labelled sealed containers until required for further laboratory analysis.

2.2 Plants Extraction

The pulverized plant materials will be exhaustively extracted using ultra sonication method. The method was adopted from Zhijian *et al* (2016) with little modification. The 20g of powdered sample and 60mL of solvents (Hexane and Methanol) were added into the extraction tube and placed into the ultrasonic instrument at 30^0C for extraction. The electric power and generators frequency is fixed at 200W and 40 kHz respectively and the extraction were carried out for 40minutes. Then, the tubes were centrifuged at 10,000g for 10 min to remove the sediment or filtered through muslin clothes. The supernatant was separated for further analysis

2.3 Phytochemical Screening

Phytochemical analysis of the extracts was carried out according to the methods of Sofowora (1993) Trease and Evans (1989) using slight modification.

2.4 Antioxidant studies

The antioxidant activities of *Acacia nilotica* (pods and seeds) were determined on the basis of their scavenging activity of stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. From the calibration curves obtained from different concentration of the extracts, the inhibitory concentration (IC_{50}) was determined. The IC_{50} value represents the concentration of sample required to scavenge 50% of the DPPH free radicals (Gupta *et al*., 2003).

2.5 Antimicrobial Studies

The crude extracts were screened for antibacterial and fungal activities using agar in-well diffusion technique with little modification as described by El-Mahmood (2009). Different bacterial and fungal strains namely *Staphylococcus aureus*, *Pseudomonas auregenosa*, *Streptococcus pyogenes* and *Salmonella typhi*, *Candida albica* were used for testing antibacterial and antifungal activity. Clinical isolates of bacteria were obtained from the Department of Microbiology University of Maiduguri Teaching Hospital (UMTH).

Standardization of the cultures was carried out according to the National Committee for Clinical Laboratory Standards. Sterilized Mueller Hinton agar plates was prepared using manufacturer's standard and was inoculated with the test culture by surface spreading using sterile cotton bud or swab stick. The wells were made in each culture plates using a sterile 6 mm cork borer. Approximately, 0.1 ml of the crude extract (300, 200 and 100mls µg/ml respectively) were dispensed in each well and incubated for 24 hours at 37°C. The plates were examined for the presence of bacterial growth inhibition zones around each well. The diameter of the zones of inhibition were measured using a ruler and the results were reported in millimeters (mm).

3.0 Results

3.1 Phytochemical Screening

Table 1 shows the phytochemical component from *Acacia nilotica* pods extracted using n-Hexane and methanol.

Tannins, alkaloids, Saponins and glycosides were present while alkaloid was absent in all the extracts.

Table 1: Qualitative Phytochemical Components of *Acacia nilotica* Pod Extract

Phytochemicals	n-Hexane	Methanol
Tannins	+	+++
Flavonoids	-	-
Alkaloids	++	+
Saponins	+	++
Glycosides	+	+

Table 2 shows the presence of tannins, alkaloids and glycosides in both extracts of Acacia nilotica seed. Flavonoids was however only present in methanol extract. The results further revealed that Saponins was absent in all extracts.

Table 2: Qualitative Phytochemical Components of *Acacia nilotica* Seed Extract

Phytochemicals	n-Hexane	Methanol
Tannins	+	+
Flavonoids	-	+
Alkaloids	+	+
Saponins	-	-
Glycosides	+	+

3.2 Antioxidant Activity

Figure 1 present the UV-Absorbance at 517nm of Pods of *Acacia nilotica* Extracts of n-Hexane and Methanol.

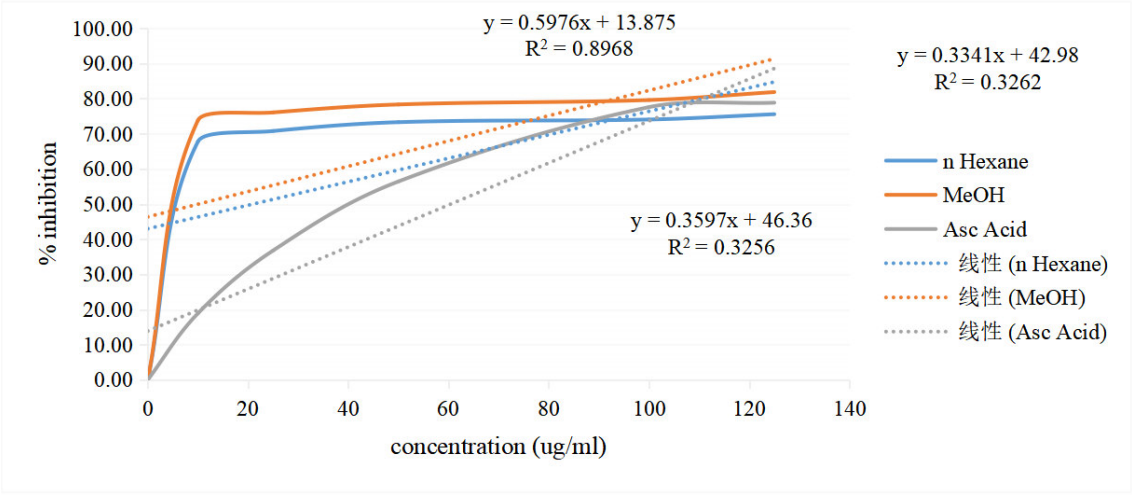


Figure 2 shows the Percentage Inhibition of Pods of *Acacia nilotica* Extracts (n-hexane and Methanol) as well as standard ascorbic acid. The results shows methanol extract to possess highest antioxidant activity, followed by n-hexane extract and finally ascorbic acid.

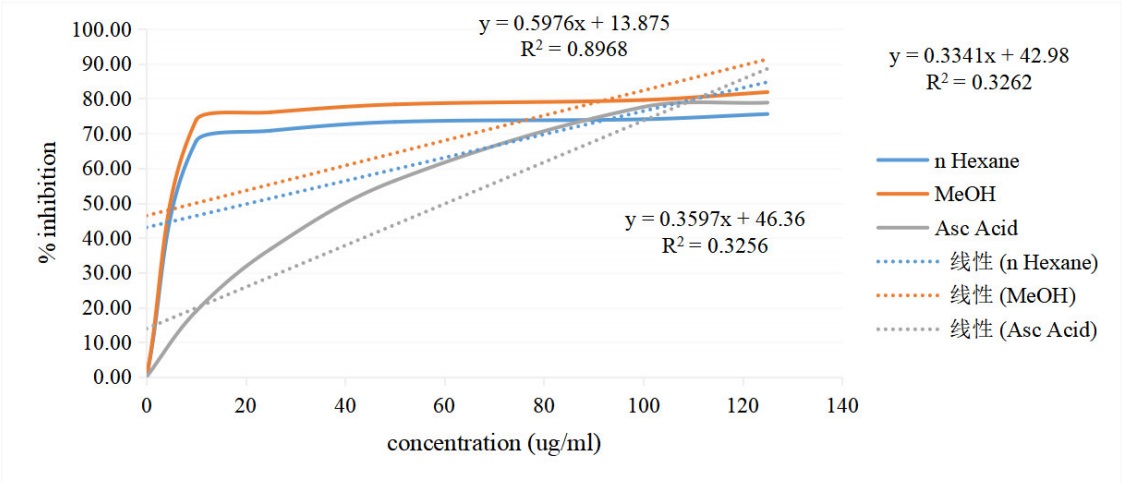


Figure 2: Percentage Inhibition of Pods of *Acacia nilotica* Extracts (n-hexane and Methanol) as well as standard ascorbic acid

The UV-Absorbance at 517nm of seeds of *Acacia nilotica* solvents extracts (n-Hexane and Methanol) was presented in figure 3.

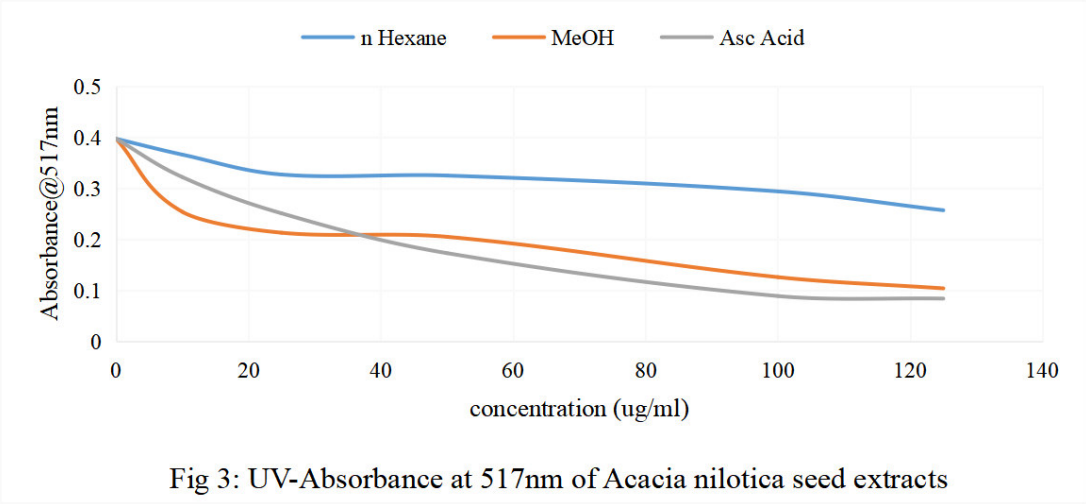
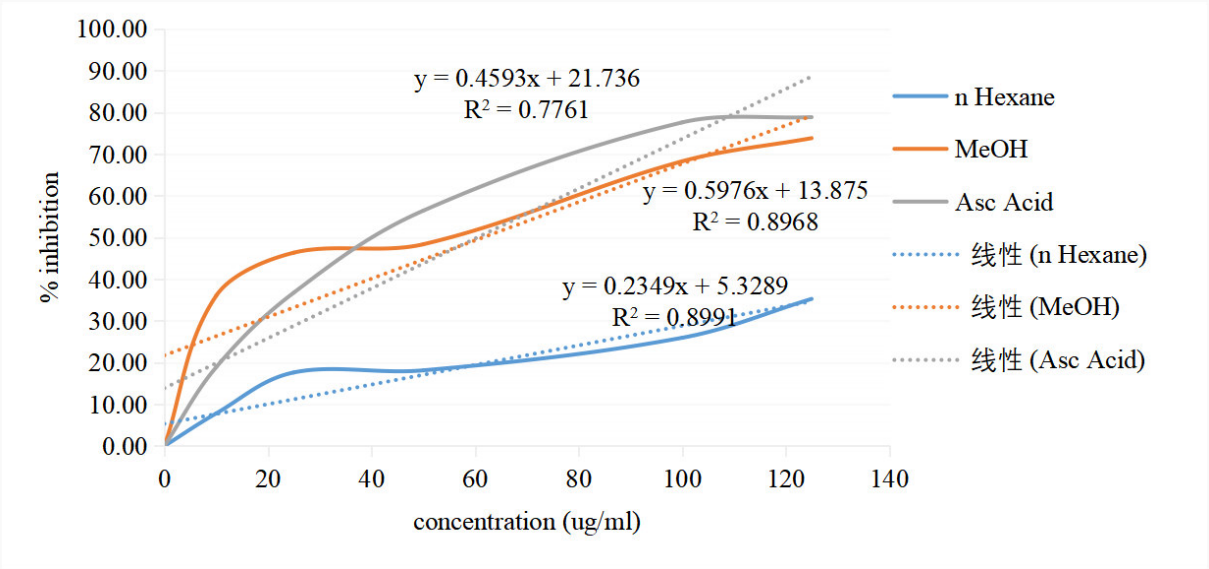


Fig 3: UV-Absorbance at 517nm of Acacia nilotica seed extracts

The Percentage Inhibition of *Acacia nilotica* seed extracts was presented in figure 4, the results shows methanol extract to have higher activity than the n-hexane extract. However, ascorbic acid shows highest activity.



3.3 Antimicrobial Studies

The minimum inhibitory concentration (MIC) and minimum inhibitory bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) of pods of *Acacia nilotica* extracts against bacterial and fungi isolates at lowest concentration was shown in table 3 below. The results indicate MIC, MBC/ MFC values from 4.15ug/ml to 5.25ug/ml for the extracts studied. In all the extracts the MIC values are equal to MBC/MFC (4.15ug/mg or 5.25ug/mg) indicating that small amounts of these extracts are needed to bring about bactericidal or fungicidal effects.

Table 3: MIC, MBC/MFC of pods of *Acacia nilotica* against some selected pathogens

Extract	Conc.(ug/ml)	*S. aureus*	*P. aeruginosa*	*S. pyogenes*	*S. typhi*	*C. albica*
n-Hexane	MIC	5.25	0	4.15	5.25	0
	MBC	5.25	0	4.15	5.25	0
	MFC	0	0	0	0	0
methanol	MIC	0	0	4.15	5.25	5.25
	MBC	0	0	4.15	5.25	0
	MFC	0	0	0	0	5.25

4.0 Discussions

Phytochemicals are bioactive compounds obtained from the plants and are widely applied in the traditional herbal medicine. These herbal medicines are used by the local people to cure the various diseases which include the major diseases such as Diabetes Mellitus, Cancer, HIV (Chothani and Vaghasiya, 2011). The present study showed the presence of tannins, alkaloids, saponins and glycosides while alkaloid was absent in both n-hexane and methanol extract from pod of *Acacia nilotica*. Screening of the seed extract revealed the presence of tannins, alkaloids and glycosides in both extracts of *Acacia nilotica* seed. However, flavonoids were only detected in methanol extract. These observations is in line with the findings of various authors who reported the presence of similar phytochemicals from the plant (Banso, 2009; Baravkar *et al*., 2008).

Flavonoids have also been reported to be potent antioxidant and free radicals scavengers capable of protecting cell membranes from damage (Wasagu and Laval, 2005). *In vitro* studies have shown that flavonoids have anti-allergic, anti-inflammatory, anti- microbial, anti-cancer and hypolipidemic effects. Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine and vinblastine). Some alkaloids have stimulant property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug (Wang *et al*., 2004).

Tannins were reported to have therapeutic uses due to their anti-inflammatory, anti-fungal, antioxidant and healing properties. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals (Sachin, 2013).

Alkaloid are often toxic to men and may have dramatic physiological activities hence they are widely used in medicine (Olaofe and Sanni, 1988).Lower dose of alkaloids mediate important pharmacological activities, such as analgesic, reducing blood pressure, killing tumour cells, stimulating circulation and respiration (Egbuna and Ifemeje 2015).

Saponins are naturally oily glycosides occurring in wide variety of plants (Applebaum *et al*., 1969) and are generally known for their bitter taste, foaming in aqueous solutions, and their ability to haemolyse red blood cells when injected into the blood stream (Birk and Peri, 1980). When eaten, they are practically nonpoisonous to warm blooded animals. However, high saponin level has been associated with gastroenteritis manifested by diarrhea and dysentery (Awe and Sodipo, 2001).

Many studies have shown oxidative stress to be associated pathophysiology of many disease including diabetes, hypertension, cardiovascular diseases and nephrotoxicity. Increased formation of free radicals and decreased antioxidant potential, leading to oxidative damage of cell components (Ziegler *et al*., 2011).

In vitro analysis of the pod and seed extracts shows a promising antioxidant activity as it compare favorably with standard antioxidant (ascorbic acid). Thus, this might be the reason why this plant is widely used in the management and/or treatment of various diseases as reported by (Kalaivani and Mathew, 2010; Meena *et al*., 2006; Singh *et al*., 2009).

5.0 Conclusion

The research demonstrated *Acacia nilotica* seed and pods extract to possess strong anti-oxidant and anti-microbial properties which might be due to the presence of various secondary metabolites like tannins, saponins and flavonoids.

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