



Phytochemical Screening and Antifungal Activities of Cashew (*Anacardium occidentale* Linn.) Leaves Extract on Some Fungal Isolates

I. Y. Tafinta^{1*}, N. H. Okoye¹, U. S. Batagarawa¹, I. I. Hamma² and M. Abubakar³

¹Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, P.M.B. 2346, Sokoto State, Nigeria.

²Department of Science Laboratory Technology, Gombe State Polytechnic, Bajoga, Nigeria.

³Yobe State Environmental Protection Agency (YOSEPA), Damaturu, Yobe State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author IYT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NHO managed literature search. While authors USB, IIH and MA checked the overall write-up, improved the literature and managed the analyses of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The study on qualitative phytochemical screening and antifungal activities was evaluated on Cashew (*Anacardium occidentale* Linn.) leaf extracts using standard procedures.

Objectives: With the view of evaluating its secondary metabolites and also assessing its antifungal activities.

Methodology: The antifungal activities of the leaves extracts (aqueous and ethanol) were carried out using agar incorporation method at varying concentrations (20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL). The aqueous and ethanolic extracts were tested against *Aspergillus niger* and *Rhizopus stolonifer* (isolated from street vended sliced fruits).

Results: The phytochemical screening revealed that *A. occidentale* leaf extracts (aqueous and ethanolic) contained; Tannins, flavonoids, alkaloids, Cardiac glycosides, Glycosides, Saponins glycosides, Saponins, Steroids and Volatile oils with the exceptions of Anthraquinones and Balsams. The result shows that aqueous extracts has no significant inhibitory activity when compared to the

*Corresponding author: E-mail: tafintaiy08@gmail.com, ibrahim.yusuf@udusok.edu.ng;

ethanolic extracts against *A. niger* ($p = 0.05$). The highest mean zone of inhibition (38.00 ± 5.00 mm) was observed at 100 mg/mL concentration of the aqueous extract and the lowest mean zone of inhibition (12.67 ± 2.51 mm) was observed at 20 mg/mL concentration of the ethanol extracts against *A. niger* while *R. stolonifer* were highly resistant to both extracts. The minimum inhibitory concentration of the extract (MIC) was observed at 20 mg/mL for both extracts.

Conclusion: Thus, the study showed that *A. occidentale* could be a possible source of obtaining new and effective drugs.

Keywords: Phytochemicals; extracts; antifungal; incorporation; cashew.

1. INTRODUCTION

Plants are important in our everyday existence as they provided us with food for nourishments, the oxygen we breathe and serve as raw materials for many industrial and domestic products such as; food, clothes, foot wears and many others [1]. In addition to serving humanity, plants also help in the provision of shelter, preventing and curing ailments [2]. Plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects [3]. Medicinal plants represent rich source of antimicrobial agents, thus, they are used in different countries as a good source of many potent and powerful drugs [4]. According to the world health organization [5] a medicinal plant is any plant which contains substances that can be used for therapeutic purpose in one or more of its organs or substances which are precursors for the synthesis of useful drugs. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. The plant parts used includes; the root, leaves, stem, bark, flower, exudates, fruits, twigs and modified plant organs. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. Plant species have been used in the formulation of various proprietary medicines and it is therefore important to determine the active chemical components of such plants in order to develop more effective drugs [6]. *Anacardium occidentale* L. (Cashew plant) is a tropical evergreen tree that produces the cashew seed apple. It is said to originate in Latin America, specifically north-eastern Brazil [7]. Its water-resistant wood is used for boats and ferries, while the resin, in addition to having industrial uses, is used as an expectorant, cough remedy and insect repellent [8]. *Anacardium occidentale* (Linn.) is an important tropical nut tree that

belongs to the family Anacardiaceae, which includes about 75 genera and 700 species among which the well economically known ones are mango and pistachio [9].

The leaves and bark of cashew have bactericidal and germicidal activities [10]. They also help to stop dry secretion, increases libido, and reduce fever, blood sugar and pressure [11]. In western Nigeria young leaves are used for arthritis and other inflammatory conditions. In some parts of Nigeria, bark, roots and leaves are traditionally used for the treatment of numerous diseases such as; allergy, cough, stomach ache, diarrhea, skin infections and others [12]. In the Southeastern part of Nigeria, the leaf extracts is used to bath malaria patients [13]. An infusion of the stem bark and leaves of the plant is used as a remedy for tooth ache and sore gums while the astringent bark is given for severe diarrhea and thrush [14]. The cashew apple has higher vitamin C content than oranges and mangos thus; they are used in the treatment of cough and scurvy [10]. Cashew apple is also anti-scorbutic, astringent and diuretic, and is used for cholera and kidney troubles [8]. The cashew kernel (nuts) are rich in protein, carbohydrate, fat (heart friendly monounsaturated fatty acid), oil, manganese, magnesium, zinc, potassium, copper and iron for preventing deficiency diseases and serving as antioxidants [15].

Industrially, the bark contains an acrid sap of thick brown resin, which becomes black on exposure to air and is used as indelible ink in marking and printing linens. The resin is also used as a varnish, a preservative for fishnets and a flux for solder metals. The stem also yields an amber-colored gum, which is partly soluble in water. This gum is used as an adhesive (for woodwork panels, plywood, bookbinding), partly because it has insecticidal properties [16].

Morphologically, the structure of cashew tree makes it a foremost tree for reclaiming land area to enhance productivity, through the prevention of desertification and soil erosion [17]. Pressed

cake from cashew nut kernel serves as food for animal feed [10]. Their high biological activities have been attributed to their high tannin contents [18]. Cashew leaf extract was found to contain high amounts of flavonoid and phenolic compounds which exhibited anti-hypertensive activity [19].

2. MATERIALS AND METHODS

2.1 Sample Collection and Processing

The sample collection and processing were carried out according to the method described by [20]. Fresh leaves of *Anacardium occidentale* (cashew leaves) were collected in the evening by 5 pm from Fadama at Moore along Illela road, Sokoto State in July, 2016. The leaves were washed and dried over a period of two weeks. The dried samples were milled into powder by pounding manually with a clean mortar; it was then sieved to obtain a fine powder and was stored in sterile cellophane bags in a cool dry place till further use.

2.2 Extraction of Plant Material

The sample was extracted by using the method of [21]. The grounded leaves of *A. occidentale* obtained above were weighed (100 grams for each solvent) and dissolved into 1000 mL of sterile distilled water for aqueous extract and 95% of ethanol for ethanol extract respectively in a conical flask and was covered with cotton wool and aluminum foil to avoid contaminations. The conical flasks containing the extracts were agitated and allowed to stand at room temperature for 48 hours after which they were filtered using a muslin cloth. The filtrate was then evaporated into dryness in an oven at 37°C to yield crude extracts. The crude extracts obtained were preserved in a sterile container at room temperature until further use.

2.3 Qualitative Phytochemical Screening

The identification of chemical classes present in extracts of *A. occidentale* leaves is based on the observation of color change or formation of precipitate after the addition of specific reagents. The major secondary metabolites classes such as steroids, and flavonoids, alkaloids, Saponins and glycosides were screened according to the common phytochemical methods described by [22,23].

Test for Flavonoid: 1 mL of 10% sodium hydroxide was added to 3 mL of the extract. If an

intense yellow color was produced in the plant extract, which became colorless on addition of a few drops of dilute acid indicates the presence of flavonoids compounds.

Tests for Tannins: 2 drops of 5% ferric chloride solution was added to 3 mL of the extract and colored produced was noted. Condensed tannins usually give dark green color while hydrolyzed tannins give blue-black color.

Test for Saponins: Saponins were detected using the froth test. 5 mL of the extract was added to 5 mL of sterile distilled water in a test tube. The test tube was then shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Presence of honeycomb froth indicates positive result for Saponins.

Test for Glycosides: 2.5 mL of dilute sulphuric acid was added to 5 mL of the extract in a test tube and was heated in boiling water for 15 minutes. It was allowed to cool and was neutralized with 10% sodium hydroxide and then 5 mL of Fehling's solution was added. A brick-red precipitate was observed which indicates the presence of glycosides.

Test for Alkaloids: 2 mL of the extract was stirred with 2 mL of 10% hydrochloric acid in a test tube. 1 mL was treated with few drops of Wagner's reagent and a second 1 mL portion was treated similarly with Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitate which gives preliminary evidence for the presence of alkaloids.

Test for Steroids: 0.5 mL of the extract was dissolved in 2 mL of chloroform. 2 mL of sulphuric acid was carefully added to the mixture to form lower layer. A reddish brown color at their interface indicates the presence of a steroid ring.

Tests for Saponins Glycosides: 2 mL of the extract was added to 2.5 mL of Fehling's solution A and B. A bluish green precipitate showed the presence of Saponins glycoside.

Test for Cardiac Glycosides: 2 mL of 3.5% ferric chloride solution was added to 3 mL of the extract and was allowed to stand for a minute. Then 1 mL of concentrated sulphuric acid was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at their interface indicates the presence of cardiac glycoside.

Tests for Balsams: The extract was mixed with equal volume of 90% ethanol, 2 drops of alcoholic ferric chloride was added to the mixture. Formation of a dark green color indicates the presence of balsams.

Test for Anthraquinones: 2 mL of the plant extract was shaken with 10 mL of benzene and 5 mL of 10% ammonia solution was added. The mixture was shaken and the presence of a pink, red, or violet color in the lower phase indicates the presence of anthraquinones.

Tests for Volatile Oils: 1 mL of the extract was mixed with dilute hydrochloric acid. The formation of white precipitate indicates the presence of volatile oil.

2.4 Test Organisms

The fungal species; *Aspergillus niger* and *Rhizopus stolonifer* isolated from street vended fruits, were obtained from the stock culture of Mycology Laboratory, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto.

2.5 Media Preparation

The media used was potato dextrose agar (PDA). This media was prepared according to the manufacturer's instructions. Thirty nine grams (39 g) of PDA was dissolved in one thousand milliliters (1000 mL) of distilled water in a conical flask and was covered with cotton wool and Aluminium foil. The mixture was heated on a hot plate until it was dissolved completely and was sterilized in an autoclave at 121°C for 15 minutes.

2.6 Preparation of Extract Concentrations

100 mL of distilled water was sterilized in an autoclave at 121°C for 15 minutes and was allowed to cool. To prepare a stock solution of the extracts, 10 grams (10,000 mg) of *Anacardium occidentale* crude extracts obtained above was dissolved in 100 mLs of sterile distilled water in a beaker and was stirred with the aid of a glass rod to ensure proper dilution. The solution was then transferred into a volumetric flask, covered and was labeled as 100 mg/ mL of *A. occidentale* leaves extracts. Various concentrations (20 mg, 40 mg, 60 mg, 80 mg and 100 mg) of *Anacardium occidentale* leaf extract (aqueous and ethanol extract) were prepared from the stock solution using dilution method [24]. Formula used for calculating dilution method was $C_1V_1=C_2V_2$. Where; C_1 =

concentration before dilution, V_1 = volume before dilution, C_2 = concentration after dilution and V_2 = volume after dilution.

2.7 Antifungal Activity Test

Antifungal activity test was carried out using the agar incorporation method. 20 mL of the prepared PDA media was mixed with 5 mL of the extract solution for each varying concentrations (20 mg/ mL, 40 mg/ mL, 60 mg/ mL, 80 mg/ mL and 100 mg/ mL) in a conical flask and was poured into sterile petri dishes. 20 mL s of the prepared PDA media was poured into a sterile petri dish without the extracts which serves as the control. About six plates were prepared for both organisms. The petri dishes containing the media were allowed to solidify before inoculating the test organisms.

The test organisms were introduced into the plates containing solidified media aseptically with the aid of a sterile wire loop. The wire loop was sterilized before introducing the organism in each plate by placing it in a bunsen burner until it turns red, then it was removed and waved in the air for it to cool before picking the organism from the pure culture. Three replications were maintained for each concentration. The plates were then incubated at $28 \pm 2^\circ\text{C}$ for five days in an incubator. The antifungal activity was evaluated by measuring the relative growth of fungus in the plates containing varying concentrations (treatments) and compared to the growth in the control plate. The minimum inhibitory concentration of the extract was observed as the lowest concentration of the extract which inhibited visible growth of the fungus [25].

2.8 Statistical Analysis

The experimental results were expressed as mean plus or minus standard deviation (mean \pm SD) of triplicates values. Student paired t-test was used for the evaluation of difference between the aqueous and ethanol extracts while one-way analysis of variance (ANOVA) was used to evaluate the differences between the concentrations for each extracts.

3. RESULTS

3.1 Phytochemical Screening Results

Table 1 presents the qualitative phytochemical screening carried out on the leaf extracts of *Anacardium occidentale*. It showed that

alkaloids, flavonoids, Saponins, tannins, glycosides and steroids were present while balsams and anthraquinones were not detected.

3.2 Antifungal Activities of the Extracts

The comparison between aqueous and ethanolic extracts of *A. occidentale* leaves using paired student t-test showed that, there was no significant difference between the two extracts at $p= 0.05$. The results of antifungal activity of both aqueous and ethanol leaves extracts of *A. occidentale* at various concentrations (0 mg/ mL, 20 mg/ mL, 40 mg/ mL, 60 mg/ mL, 80 mg/ mL and 100 mg/ mL) on *A. niger* and *R. stolonifer* are shown in the Tables below.

Table 2, Represents the results of the effect of *A. occidentale* extracts on the growth of *Aspergillus niger* and *Rhizopus stolonifer*. From the table, it was observed that the antifungal activity of the extracts (aqueous and ethanol) increased with increase in extract concentration. The reduction in the growth of fungi ranged from 12.67 ± 2.51

mm to 38.00 ± 5.00 mm. It was observed that the aqueous extract had higher activity than the ethanol extract against *A. niger* while both extracts had no activity on *R. stolonifer*. *Aspergillus niger* had the lowest zone of inhibition (12.67 ± 2.51 mm and 13.00 ± 1.00 mm) at the concentration of 20 mg/ mL for ethanolic and aqueous extract and highest zone of inhibition (32.67 ± 3.05 mm and 38.00 ± 5.00 mm) at the concentration of 100 mg/ mL.

Table 3, Represents the comparison between the means of aqueous and ethanol extracts of *A. occidentale* against *A. niger*. It was observed that there was significant difference between concentrations of each extract at $p < 0.05$. The table also indicated that between the concentrations of 20 mg/ mL and 40 mg/ mL, 100 mg/ mL and 80 mg/ mL and 60 mg/ mL and 80 mg/ mL of aqueous extract there was no significant difference. While for the ethanolic extract, there was no significant difference between the concentrations of 20 mg/ mL and 40 mg/ mL, 60 mg/ mL and 80 mg/ mL.

Table 1. Qualitative phytochemical test of *A. occidentale* leaves extract

Phytochemical compounds	Results
Alkaloids	+
Anthraquinones	ND
Balsams	ND
Cardiac glycosides	+
Flavonoids	+
Glycosides	+
Saponins glycosides	++
Saponins	++
Steroids	++
Tannins	+++
Volatile oils	+

Key: + (present), ++ (moderately present), +++ (adequately present) and ND (not detected)

Table 2. Effect of different concentrations of *Anacardium occidentale* leaves extract (aqueous and ethanol) against *A. niger* and *R. stolonifer*

Extracts	Concentrations (mg/mL)	Zones of inhibition (mm)	
		<i>A. niger</i>	<i>R. stolonifer</i>
Aqueous	20	13.00 ± 1.00	-
	40	16.33 ± 6.11	-
	60	27.00 ± 4.58	-
	80	33.00 ± 3.61	-
	100	38.00 ± 5.00	-
Ethanol	20	12.67 ± 2.51	-
	40	15.00 ± 4.00	-
	60	25.33 ± 6.43	-
	80	26.00 ± 7.62	-
	100	32.67 ± 3.05	-
Negative control (without extract)	0	-	-

Each value represents the mean \pm standard deviation of triplicate readings while (-) shows no inhibition

Table 3. Comparison between the mean zones of inhibition of *A. occidentale* leaves extracts (aqueous and ethanol) against *A. niger*

Concentrations (mg/mL)	Mean zone of inhibition (mm)	
	Aqueous extract	Ethanol extract
0	0.00±0.00 ^d	0.00±0.00 ^d
20	13.00± 1.00 ^c	12.67±2.51 ^c
40	16.33± 6.11 ^c	15.00± 4.00 ^c
60	27.00± 4.58 ^b	25.00± 7.00 ^b
80	33.00± 3.61 ^{ab}	26.00± 5.29 ^b
100	38.00± 5.00 ^a	32.67± 3.05 ^a

Each value represents the mean± standard deviation of triplicate readings
Mean values in the same column with different superscripts are significantly different

4. DISCUSSION

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobial agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism [26]. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds. Therefore, medicinal plants are finding their way into pharmaceuticals, nutraceuticals and food Supplements [27].

In this research, the qualitative phytochemical screening carried out on *Anacardium occidentale* revealed the presence of Alkaloids, Cardiac glycosides, Flavonoids, Glycosides, Saponins glycosides, Saponins, Steroids, volatile oils and Tannins but with exceptions of Anthraquinones and Balsams presented in Table 1. [28] had earlier reported the presence of tannins, alkaloids, Saponins and flavonoids in *A. occidentale* leaves. Thus the antimicrobial activity of the extracts on the test organism may be due to the presence of the above phytochemical components. The presence of these bioactive components is a strong indication that *Anacardium occidentale* has medicinal potentials [29].

Also in this study, aqueous and ethanol extract of *Anacardium occidentale* leaves were evaluated for their antifungal activity against *Aspergillus niger* and *Rhizopus stolonifer*. The results obtained showed that both extracts were active against *A. niger* while *R. stolonifer* were highly

resistant to the extracts. The antifungal activity of the aqueous and ethanolic extracts of *Anacardium occidentale* showed significant variations against *A. niger*. Among the two extracts tested, aqueous extract had greater antifungal potential than the ethanolic extracts. The minimum inhibitory concentration (MIC) was observed at the concentration of 20 mg/ mL for both extracts of *A. occidentale* leaves. The largest mean zones of inhibition observed against *A. niger* are 38.00± 5.00 mm for aqueous extract and 32.67± 3.05 mm for ethanolic extract while the lowest zone of inhibition were (13.00± 1.00 mm and 12.67± 2.51 mm) for aqueous and ethanolic extracts respectively. A phytochemical screening analysis on *Anacardium occidentale* leaves have showed the presence of high concentration of tannins in the aqueous extract and its absence in the alcoholic leaf extract [1]. This could probably account for the effective action of aqueous form compared to the ethanolic extract in the inhibitory effect. On the contrary, [11] evaluated the antimicrobial activity of aqueous and ethanolic extracts of the leaves of *Anacardium occidentale* and found ethanolic extract to be highly active against selected microbes like; *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherchia coli*, *Candida albicans* and *Aspergillus niger* than the aqueous extract. From this study conducted, it indicated that *Anacardium occidentale* extracts have broad inhibitory activities to pathogenic microorganisms and promising to act as potential antibacterial and antifungal agents from natural plant sources.

5. CONCLUSION

Base on the result obtained, It can be concluded that *Anacardium occidentale* leaves extract showed antifungal activity against *A. niger* and contains phytochemicals which may serve as source of new and effective drugs. Therefore, the

study supports the use of *A. occidentale* in traditional medicine to treat various ailments like stomach pain, wound infections, toothache and Asthma as practiced in the past.

6. RECOMMENDATIONS

1. More research should be carried out on *A. occidentale* so as to establish fully its usefulness to humanity as a source of drugs for the alleviation of illnesses caused by micro and macro organisms.
2. Since *A. occidentale* had been found to have medicinal uses, it is important to conserve it in order to ensure its continuity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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