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# Isolation and Characterization of the Chemical Constituents of *Anacardium occidentale* Cracked Bark

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## ABSTRACT

The cracked bark of *Anacardium occidentale* were dried under ambient conditions, chopped into bits and the ethanolic extract of the cracked bark was obtained by cold extraction. Phytochemical screening was conducted to identify the types of secondary metabolites present using standard procedures. The profiles of the chemical constituents present were established using Thin Layer and Column Chromatography methods. Thus, pure chemical constituents were isolated from the cracked bark of *Anacardium occidentale*. The isolated compounds were characterized using FT-IR and their structures determined using data obtained from GC-MS spectrum.

**Keywords:** *Anacardium occidentale*, ethanolic, cracked bark, phytochemical, isolated, characterized.

## 1. INTRODUCTION

Cashew *(Anacardium occidentale)* is a tree that grows up to 15m in height with thick tortuous trunk and woody branches. It belongs to the family anacardiaceae, native to Brazil and it is distributed throughout tropical countries such as Nigeria, Kenya, Tanzania, Mozambique[1,2]. *Anacardium occidentale* is commonly called cashew in English and in the major Nigerian languages: Hausa, Ibo and Yoruba, it is called 'Kashu', 'Okpokpo'and 'Kaju' respectively. The cashew tree produces many products. The bark, leaves and shell oil of *A.occidentale* have important medicinal values and industrial applications in the plastic and resin industries based on its phenol content. The nut has international appeal and high market value as a food source, especially in the beverage industry[3].

*A. Occidentale* is a multipurpose tree whose leaves, stems and bark extracts have been used extensively for the treatment of diarrhea, dysentery and colonic pain. There are reports that it possesses anti-diabetic, anti-inflammatory, antimicrobial and analgesic properties[4,5]. The antimicrobial activity of ethanolic extract of *A. Occidentale* leaves was attributed to2-hydroxy-6-pentadecylbenzoic acid**[1]** and other compounds such as tatrols and tannins which are some of the chemical constituents identified in the ethanolic extract[6].

Phytochemical analysis of *A. occidentale* reveals a rich variety of secondary metabolites. The ethanolic extract of *A. occidentale* L. nuts contains various phytochemical compounds such as triterpenoids, phenolics and volatile oils. Ethylacetate extract exhibited a different combination of phytochemicals: phenolics, volatile oils, xanthoprotein and carbohydrates. Acetone extract contained compounds like triterpenoids, phenolics, volatile oils, flavonoids, xanthoprotein and carbohydrates[1,7].

Several studies have been carried out to isolate natural products from various parts of *A. occcidentale*. 2-hydroxy-6-pentadecylbenzoic acid**[1]** and 2,6-dihydroxybenzoic acid **[2]**have been isolated from the cashew apple[8]. Other compounds isolated include myricetin**[3]**, quercetin**[4]**, kaempferol**[5]**, rhamnetin**[6]**[9], cyaniding **[7]**, peonidin**[8]** and delphinidin**[9]** were also isolated [2].

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2-hydroxy-6-pentadecylbenzoic acid**[1]**, cardanol**[10]** and salicyclic acid **[11]**were isolated from the hydro-ethanolic extract of *A. occidentale* nuts[10].

Also isolated from the ethanolic extract of *A. occidentale* flower were ethyl gallate**[12]**, hyperoside (quercetin 3-galactoside) **[13]**. And from the ethanolic extract of the tender leaves, β-sitosterol**[14]** was isolated[11].

The research was carried out to study the chemical constituents of *A. occidentale* and discuss their relevance to the medicinal uses of the plant by isolation of the chemical compounds present as well as their characterization.

## 2. MATERIALS AND METHODS

### 2.1 *Collection and identification of plant materials*

*Anacardium occidentale* cracked bark was collected from University of Ilorin Botanical Garden, Nigeria. The plant samples were collected from four different trees, identified and documented at the Herbarium of the Department of Plant Biology, University of Ilorin.

### 2.2 *Extract preparation*

The cracked stem bark was pulverized and dried under ambient conditions. 600 grams of the plant material was extracted with ethanol for 72 hours and the resultant crude extract obtained was filtered. The crude extract was concentrated under reduced pressure using a rotary evaporator to obtain a solvent-free ethanol extract.

### 2.3 Phytochemical analysis

Qualitative phytochemical screening was carried to determine the presence of alkaloids, saponins, phenolics, flavonoids, steroids and terpenoids using the standard literature procedure [12].

### 2.4 *Thin layer chromatography*

Thin layer chromatography of the cracked bark was carried out to identify the unique distinguishing chemical constituents present in the cracked bark. Commercial thin layer chromatographic plate (Merck, Germany) was used. The solvent used was dichloromethane. Thin Layer Chromatograms were viewed under the UV lamp at the long wavelength of 366nm.

### 2.5 *Column chromatography*

The crude extracts were fractionated using Si-gel column chromatography. n-hexane and dichloromethane were used as eluents. Fractions obtained were collected in 50mL flasks. Identical fractions were pooled based on their TLC profiles thus resulting in 5 combined fractions. Further purification using preparative thin layer chromatography was carried out on fractions that were impure.

### 2.6 *Preparative thin layer chromatography*

Self-coated preparative thin layer chromatography plates were used to isolate and purify the chemical components. The plates were coated with slurry of silica gel mixed with appropriate amount of binder (calcium sulphate) and activated at $103^0$c for 15mins in a drying oven.

### 2.7 *Spectroscopic Analyses*

The isolates were characterized using data obtained from Fourier-Transform Infrared (FTIR) Spectroscopy and Gas Chromatography-Mass Spectroscopy (GC-MS).

#### 2.7.1 *Infrared Spectroscopy*

The infrared spectroscopic analyses of the isolates were carried out so as to know the different functional groups present in the isolated compounds. The infrared spectra were recorded on Shimadzu 8400s (Schimadzu Corporation, Kyoto Japan) Fourier Transform-Infrared (FT-IR) Spectrophotometry using KBr pellet.

#### 2.7.2 GC-MS Analysis

The analysis was done on Agilent 7890A GC/MS equipped with a Quadrupole Mass Spectra Detector and an Auto-sampler. GC-MS system settings are as follows; $200^0$C, interfaced temperature, $250^0$C, solvent cut time; 2.50 min; relative detector mode, ACQ mode; Scan; start time – end time; 3.00 min – 56.00 min, event time,0.50 sec; scan speed, 1428.

## 3. RESULTS AND DISCUSSION

### 3.1 *Phytochemical analysis*

The ethanolic extract of *Anacardium occidentale* cracked bark showed various phytochemicals like phenolics, flavonoids, triterpenoids and saponins. Alkaloids and saponins were not detected (Table 1). This agrees with the results of Fazali *et al*., (2011)[13].

### 3.2 *Column Chromatography of the Cracked Bark Extract*

A total of seventy-one fractions were collected from the column chromatography fractionation of the cracked bark crude extract (Si-gel). Combined fractions 29-39 was purified using PTLC.

### 3.3 *Infrared Spectra of Isolated Compounds*

From the IR spectra of the isolated compounds, the bands observed are summarized in table 2.0.

**F29-29/Z2**

The O-H stretching band at 3444 $cm^{-1}$ is of an alcohol. The O-H can be said to be associated. The C-H stretching at 2929 $cm^{-1}$, 2854 $cm^{-1}$ corresponds to that of an aliphatic C-H. The C-O absorption peak for alcohol appeared at 1199 $cm^{-1}$ while the O-H bend occurred at 1219 $cm^{-1}$, 1265 $cm^{-1}$.

**F29-39/Z1**

The O-H stretching band at 3423 $cm^{-1}$ is that of an alcohol. The C-H stretching at 2926 $cm^{-1}$, 2854 $cm^{-1}$ corresponds to that of an aliphatic C-H. The C-O absorption peak for alcohol appeared at 1097, 1084 $cm^{-1}$ while the C-H bends occurred at 1421$cm^{-1}$, 1442 $cm^{-1}$, 1479 $cm^{-1}$.

### 3.5 GC-MS Analyses Results

**F29-29/Z2**

Three main peaks were highlighted in the GC-MS (Table 3.0).The 3 major peaks corresponds to three different compounds including fatty acids and fatty acid esters. These peaks according to NIST Library matching are cyclohexane carboxylic acid, decyl ester (51.19%, RT = 53.434) and n-hexadecanoic acid (34.22%, RT = 45.847), all constituting 87.65% of the total.

n-hexadecanoic acid, probably responsible for the yellow color of the isolate [14], has been reported to be among the fatty acids which possess antibacterial and antifungal activity [15,16]. It may thus be implicated as part of the agents responsible for the bactericidal activity of the ethanolic extract of *A. occidentale* bark against enterotoxin producing bacteria [4]. It might also be partially or wholly responsible for the antifungal properties exhibited by the ethanolic extract against *A. flavus, A. fumigatus, A. niger, curvalaria sp* and *Fusarium sp*[1].

The 17-octadecynoic acid though not a major component of the isolate, has been reported to possess antihypertensive properties[17].

A unique and major component of the isolate proposed by the NIST library of the GC-MS is cyclohexanecarboxylic acid, decyl ester (MF 268). However from the mass spectra, the molecular formula of the matched compound is 281 while its base peak is m/z 129 which is different from the proposed compound with a base peak of m/z 57 characteristic of cyclic alcohols. The reason for this could be due to the possible presence of a substituent on the cyclohexane ring which may have been earlier fragmented. The proposed structure for this compound **(100)** and its fragmentation pattern are given in figure 4.

**F29-39/Z1**

Five compounds were observed in the GC-MS as indicated in table 6.0. The MS spectra data in table 6.0 showed 5 peaks corresponding to five different compounds. This infers the isolate is a mixture of compounds. The compounds present include 2-trifluoroacetoxydodecane (22.76%, RT = 43.032), oleic acid (18.21%, RT = 48.199), 1-cyclohexylnonene (7.03%, RT = 48.365), octadecanoic acid, 2,3-dihydroxypropyl ester (45.06%, RT = 49.052) and 3-[(trimethylsilyl)oxy]-17-[o-(phenylmethyl)oxime]-(3α,5α)-androstane-11,17-dione (7.0%, RT = 53.560). 2-trifluoroacetoxydodecane and Octadecanoic acid, 2, 3-dihydroxypropyl ester are the major compounds present as they constitute 67.82% of the total. 2-trifluoroacetoxydodecane, one of the major constituents has been reported to possess antitumor activity against murine mammary adenocarcinoma [18]. Octadecanoic acid, 2, 3-dihydroxypropyl ester also one of the main components of the isolate, has been reported to possess therapeutic activities which include antioxidant, hepatoprotective, antihistaminic, hypocholesterolemic and antieczemic activities. Its antioxidant activity is possibly evident in thereported antioxidant property of the fatty acid octacosanoic acid[19].

A unique constituent of this isolate is the androstane steroid 3-[(trimethylsilyl)oxy]-17-[o-(phenylmethyl)oxime]-(3α,5α)-androstane-11,17-dione. Although it is not a major constituent of the isolate, it is significant because no androstane steroid has been reported to be isolated from any part of *Anacardium occidentale* as at the time of this report. Furthermore, the structure proposed by the NIST library has been modified due to the low percentage matching between its mass spectra and the compound's mass spectra. The base peak of the library suggestion is m/z 91 (MF = 381) while that of the matched compound is m/z 207 (MF = 341), hence the need to modify the structure to obtain a compound whose spectra matches that obtained from the GC-MS.

The structure and fragmentation pattern of the modified androstane steroid proposed are shown in figure 6 below. It is a hydroxylimine steroid. It is noteworthy that androstane steroids have been reported to have potentials in: alleviating stress, anxiety, mood disorders, seizures, depression; treatment of drug and alcohol abuse, memory, premenstrual disorders, and neural system damage [20].

## 4. CONCLUSION

The cracked bark of A. occidentale was found to contain of fatty acid esters, the notable one being 5-methylbut-2-en-1-yl 3-hydroxy-5-methoxy cyclohexane carboxylate. Also noteworthy is a unique new and rostane steroid derivative being reported for the first time in *Anacardium. occidentale*. These are compounds which could serve as new lead compounds with promising biological activities.

## 5. AKNOWLEGDEMENT

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