KIU Journal of Humanities Copyright©2020
Kampala International University ISSN: *2415-0843*; 5(2): 405–416

Phytochemical Composition and Antimicrobial Activity of African Walnut (*Tetracarpidium conophorum*) Oil on Selected Microorganisms Causing Human Skin Infections

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Abstract. The development of antibiotic-resistant pathogenic strains due to the indiscriminate use of antibiotics has led to search for the use of novel natural antimicrobials in the treatment of human skin infections. The qualitative phytochemical screening and subsequent quantification revealed the presence of bioactive compounds such as alkaloids (3.87 ± 0.02 mg/100 g), saponins (4.37 ± 0.01 mg/100 g), phenol (2.32 ± 0.02 mg/100 g), glycoside (2.66 ± 0.02 mg/100 g), reducing sugar (3.04 ± 0.02 mg/100 g), moderate concentration of flavonoids (1.98 ± 0.02 mg/100 g), minute or trace amount of tannins (0.85 ± 0.02 mg/100 g) and soluble carbohydrate (0.99 ± 0.01 mg/100 g) while terpenoids and hydrogen cyanide were not detected in the nut oil. The assessment of the antimicrobial activity of oil extracted from African walnut (*Tetracarpidium conophorum- Mull. Arg*) was investigated using agar-well diffusion method against clinical isolates of *Staphylococcus aureus, Escherichia coli, Cutibacterium acne, Malassezia furfur* and *Candida albicans* which were isolated from human skin infections. The results of the investigation revealed that African walnut oil had inhibitory effect on the growth of all the test microorganisms. Significant differences ($p<0.05$) were observed in the degree of inhibition of the isolates, but non-significant variations were observed in inhibition among strains of the same species. The most pronounced inhibition as confirmed by the zones of inhibition around growing colonies was on *S. aureus. C. acnes* was inhibited moderately, growth inhibition of *E. coli* was mild. Growth inhibition by African walnut oil was not significant ($p>0.05$) between the two fungal strains. The inhibitory activity of the nut oil was observed to decrease with a decrease in concentration of nut oil in the solvents used, resulting in marked variation in the inhibitory concentration. The implication of this observation is that African walnut oil may be of medical and dermatological importance.

Keywords: African walnut oil, *Tetracarpidium conophorum*, phytochemical screening, antimicrobial activity, skin infections, growth inhibition

1. Introduction

The use of plant and its products have a long history of use in folk medicine and through the years have been incorporated into modern or western medicine and in certain systems of traditional medicine (Sen and Batra, 2012). Since ancient times, many plants species have been reported to have pharmacological properties as they posses various secondary metabolites like terpenoids, saponins, flavonoids, steroids, tannins and alkaloids which is utilized to combat pathogenic microorganisms (Chijioke *et al*., 2015; Igara *et al*., 2017).

With the development of technology along with the advancement in Science, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs (Sen and Batra, 2012). Antibiotic is one of the most significant therapeutic discoveries of the 20th century that had effectiveness against serious microbial infections. However, only very few of the well known human diseases have been treated from these synthetic drugs (Sen and Batra, 2012). This is because of the emergence of drug resistant pathogens that is the effect of indiscriminate use, incessant and misuse of antibiotics (Bello *et al*., 2013; Igara *et al*., 2017). Antibiotic resistance has increased recently and is posing a serious therapeutic problem. Plants produce a variety of compounds to protect themselves against a variety of pathogens. It is anticipated that plant extracts having different target

sites other than the antibiotics sites will be active against drug resistant pathogens (Sen and Batra, 2012). Over many decades, medicinal plants have been used as traditional treatments for numerous human diseases world-wide. Hence, numerous researchers have paid great attention to plant based traditional medicine practices and biologically active compounds or phytochemicals isolated from plant species used in herbal medicines with satisfactory therapeutic index for the development of novel natural antimicrobials (Sen and Batra, 2012; Nwaoguikpe *et al*., 2012; Chijioke *et al*., 2015).
The African walnut (*T. conophorum*) belongs to the family Euphorbiaceae (Edem *et al*., 2009). However, Ayodeji and Aliyu (2018) stated that some walnut species are found in the family Olacaceae. African walnut is similar to *Juglans regia* (L.), known as the English walnut and belonging to the family Juglandaceae (Raja *et al*., 2012; Lamichhane *et al*., 2016). African Walnut is a perennial creeping shrub that grows in temperate areas in Africa such as Cameroon, Gabon and Liberia including Nigeria (Ayoola *et al*., 2011). The immature fruits are usually green in colour but turn dark brown to black as they reach maturity (Ayodeji and Aliyu 2018). Walnuts are dry nuts which are encased in green pods. As walnut matures, the outer covering dries and falls off leaving the segment tough black shell and the white seed. The white seed nut is the edible nut (Ekwe and Ihemeje, 2013). Its range in Nigeria includes Uyo, Akamkpa, Akpabuyo, Lagos, Akure, Kogi, Ajaawa, Ogbomosho, Ibadan (Obianime and Uche, 2010; Ayodeji and Aliyu, 2018), Ife, Ekiti and Ijeshaland. it is abundant in all cocoa-producing states in Nigeria and in the southern part of Nigeria (Udedi *et al*., 2014; Nwaichi *et al*., 2017; Ayodeji and Aliyu 2018). It is commonly called African walnut because of its West African origin. *T. conophorum* is often called by different names such as awusa (Yoruba), Ukpa in Igbo, kaso or ngak in Cameroon. Walnut tree is about 40m in length and are usually harvested between July to December (Ayoola *et al*., 2011).

African walnut like many plants in Africa and other parts of the world has been proven to have numerous values including decorative, nutritive, medicinal, agricultural and industrial over the years. African walnut plant is cultivated principally for the nuts which are consumed as snacks. Many research studies have showed that walnut contain high amount of oil and is a major sources of protein, carbohydrate, fat and oils, vitamins and minerals (Ajaiyeoba and Fadare, 2006; Grace *et al*., 2016). African walnut contain uncommon fatty acids which are industrially important as they are used in the manufacture of protective coatings, dispersants, pharmaceuticals, cosmetics and a wide variety of synthetic intermediates as stabilizers in plastic formulations (Ogunwusi and Ibrahim, 2016). In addition, walnuts have been reported as a high density food because it contains valuable bio-active phytochemical compounds such as oxalates, phytates, and tannins (Ajaiyeoba and Fadare, 2006). The ability of the nuts to reduce cholesterol levels in human seem to be the heart of their health benefits, though the nut contain many other antioxidants, which helps to supports the immune system and they also possess some anticancer properties. African walnut is used in Nigeria to increase sperm counts in men (Ajaiyeoba and Fadare, 2006; Ogunwusi and Ibrahim, 2016). Decoctions and infusions made from the green nuts and leaves of walnut have been used to treat various human infections such as candidiasis, vaginitis, conjuctivities, glomerulo nephritis, cellulitis, endocarditis and other related diseases (Grace *et al*., 2016). Furthermore, African walnuts are rich in dietary omega-3 fatty acids which play a role in the prevention of some disorders including depression dementia particularly Alzheimer’s disease and the antimicrobial efficacy of the plant have been attributed to the presence of phytochemicals (Grace *et al*., 2016).

Treatment of bacterial and fungal diseases with oils from medicinal plants has been effectively carried out in Nigeria. Oil from parts of plants has been shown to exhibit antibacterial and antifungal activity against a wide range of pathogenic antibiotic resistant fungi and bacteria species (Okeke *et al*., 2001; Pawar and Thaker, 2006). Oil of plant origin has been used for various cosmetic purposes particularly in the formulation of skin and body care products. Apart from a few domestic uses of African walnut oil, local communities in Nigeria use the oil for the treatment of skin rashes and related skin infections caused by bacteria and fungi species. Although, there are no scientific justifications for this local practice, use of medicinal herbs and extracts from plant sources in the treatment of skin diseases is an age-long practice in many parts of the world (Irobi and Daramola, 1993). Treatment of skin rashes, boils, skin irritations, wounds, dermatitis and pyoderma with plant extracts is a common practice in Russia and Central Asia (Mamedov *et al*., 2005). With these claims in mind, firstly the phytochemical screening of African walnut oil was determined and secondly the antibacterial and antifungal activity of the oil on some skin flora microorganisms that have been implicated in human skin infections was investigated.

2. Materials and Methods

2.1 Collection and Identification of African Walnut

Fresh walnuts were collected from walnut trees growing in Ogun State in September through October 2016 and a final sample of about 3 kg was randomly taken. The nuts were taken to Olabisi Onabanjo University, Ago-Iwoye, Ogun State where they were authenticated by a taxonomist. Standard methods for sample processing and preparation and analytical procedures were used.

2.2 Sample preparation and processing

After harvest and identification, African walnuts were sorted, and damaged ones discarded. The nuts were washed in cold water to remove dirt adhering to the surface. After washing, manual separation of the black husk from the nuts was done. The nuts were then sundried in shade for 14 days (two weeks). Thereafter, the nuts were milled using MarlexExcella mixer/grinding machine (Amazon, UK), packed in air tight containers and kept in the refrigerator at 4°C for further processing.

2.3 Extraction of African walnut oil

The extraction was carried out using two different solvents such as n-hexane, and petroleum ether. Extraction was done separately with petroleum ether and n-hexane using the process of extraction described below. Prior to extraction, the pulverized nut samples were kept in an oven at 105 ^{o}C for 1 h to remove any moisture that may still be present. Twenty (20 g) of the dried nut sample was wrapped in a white muslin cloth and put into a porous thimble of the Soxhlet extractor. Then, 200 ml of different solvent such as petroleum ether and n-hexane of HPLC grade with boiling range of 40-60 ^{o}C was separately added. The Soxhlet coupled with a condenser and flask already filled with the set up was heated in a heating mantle at 65 °C to allow solvent boiling. In the process the solvent vapour travels up a distillation arm and flowed into the chamber housing the sample material. The extract seeps through the pores of the thimble and fills the siphon tube where it flows back down into the round bottom flask. The process was allowed to continue for 8 h until a clear solvent was obtained in the thimble chamber. At the end of the extraction, the resulting mixtures of the oil from the two solvents were separately filtered with a 10mm Syringe-driven with a filter 0.45um to remove any impurities. The solvents were further removed completely with a rotary-evaporator (Model N-1, Eyela, Tokyo Rikakikal Co., Ltd., Japan). The oil was stored in white bottles and tubes under nitrogen at 4 °C until analyzed (Figure 1). Extraction was repeated five times.

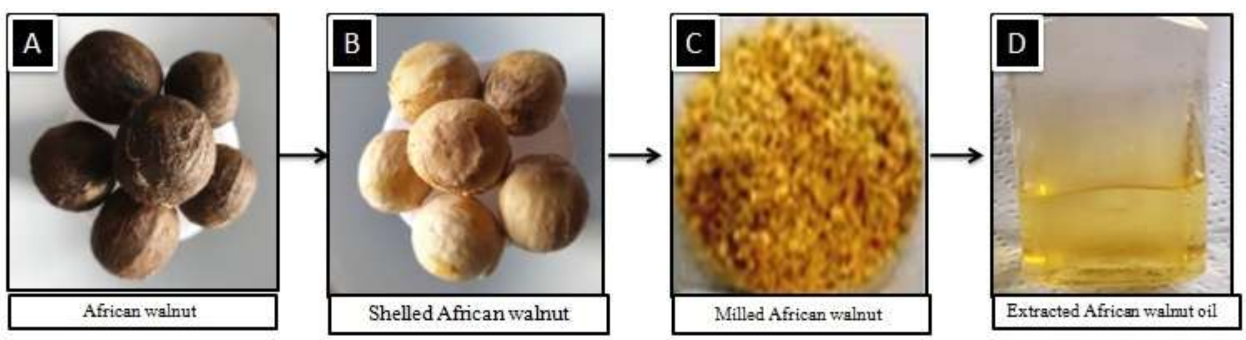

Figure 1 shows the flow chart for the solvent extraction of African walnut oil from African walnut.

2.4 Test organisms

All the strains of bacteria and fungi species used in this study were clinical isolates. The bacteria species were obtained from the Department of Medical Microbiology and Parasitology, University Teaching Hospital, (UCH) Ibadan, while the fungi species were obtained from the Federal Medical Centre, (FMC) Abeokuta. The bacterial species used included *Staphylococcus aureus, Escherichia coli, Cutibacterium acnes.* While the fungi species are *Candida albicans* and *Malassezia furfur*. Five strains of each species of organisms were used. Each of the strains has designated laboratory codes assigned to them; all the isolates were confirmed from source to be implicated in some kind of skin infection (Table 1). No information is made available on the specific biochemical and characteristics that distinguish the strains.

Table 1: Source of test microorganisms

Test microorganisms	Strain codes	Source of test microorganisms	Body parts from which the test organisms were isolated
Bacterial strains			
S. aureus	2341	UCH	Isolated from skin rash
	3411	UCH	Isolated from boil on the armpit
	3621	UCH	Isolated from skin rash
	3001	UCH	Isolated from boil on the buttocks
	1190	UCH	Isolated from boil on the face
E. coli	2333	UCH	Isolated from boil on the face
	1411	UCH	Isolated from skin rash
	1093	UCH	Isolated from skin rash
	1114	UCH	Isolated from boil on the neck
	1039	UCH	Isolated from infected skin (razor) rash
C. acnes	3143	UCH	Isolated from skin acne
	2030	UCH	Isolated from skin rash
	1190	UCH	Isolated from skin lesions associated with dermatomycoses
	1390	UCH	Isolated from skin acnes
	1102	UCH	Isolated from skin infection site on nails
Fungal strains			
M. furfur	2556	FMC	Isolated from skin infection (Pityriasis)
	2035	FMC	Isolated from skin infected lesion
	2441	FMC	Isolated from skin razor rash
	2099	FMC	Isolated from skin infection lesion
	2016	FMC	Isolated from skin infection lesion
C. albicans	1001	FMC	Isolated from skin rash
	1425	FMC	Isolated from infected skin (razor rash)
	1346	FMC	Isolated from dried scaly skin rash
	1922	FMC	Isolated from skin rash associated with superficial granulation
	2140	FMC	Isolated from skin infected with Tinea

UCH = University of College Hospital FMC= Federal Medical Centre

2.5 Re-identification of the organisms

Identification of all the isolates in this study was done in accordance with the technique of Cheesbrough, (2010). The identities of the isolates were however reconfirmed using standard morphological, biochemical methods and mycological diagnostic methods. All the bacterial test organisms were aseptically grown on 5 ml nutrient broth overnight at 37^{o}C and then subcultured onto MacConkey agar (MAC), nutrient agar (NA) and cystein lactose electrolyte deficient (CLED) medium plates to get pure cultures of the isolates. These plates were incubated at 37^{o}C for 24 hours. *Candida albicans, and Malassezia furfur* were cultured on Sabouraud's Dextrose Agar (SDA) plate or Columbia sheep blood agar supplemented with olive oil. Cultured plates were incubated at 25°C for 48 hours. Pure cultures of these isolates were identified biochemically using standard microbiological identification techniques described by Cheesbrough, (2010).

2.6 Microbiological screening

2.6.1 Preparation of culture media and its sterilization

Microbial culture media including nutrient agar (NA) and nutrient broth (NB) were purchased from Oxoid, UK. Mueller Hinton Broth (MHB), and Mueller Hinton agar (MHA) was obtained from Sigma Aldrich, Dorset, UK. All these media were suitable for the growth of bacteria cultures. For fungi culture, Sabouraud's Dextrose broth (SDB) Sabouraud's Dextrose Agar (SDA) was obtained from ThermoScientific, Hampshire, UK. All media used were prepared according to manufacturer's instruction. The media for both bacterial and fungal cultures were made up in large volumes as follows: NA, (28 g), NB, (13 g), MHB (23 g), MHA (38 g), SDB (13 g) and SDA (65 g) were separately made up in 1 litre (1000 ml) of deionised water and sterilized at 121°C for 15 minutes in an autoclave and subsequently allowed to cool after sterilization to about 45 °C (temperature at which the agars remains molten) before pouring into Petri dishes to solidify.

2.6.2 Preparation and standardization of inoculums for antimicrobial activity test

In order to effectively investigate the antibacterial activity of African walnut oil, inoculums for agar well diffusion tests were prepared in accordance with the guidelines of Clinical Laboratory Standards Institute, CLSI (formerly the National Committee for Clinical Laboratory Standards) (CLSI 2013) and Sen and Batra, (2012). Using aseptic techniques, the bacteria strains were cultured separately in 10 ml of nutrient broth and Mueller Hinton Broth overnight or to stationary phase (OD600 = >2.5). From the overnight culture, using streak plate method, a loop full of the tested strains were streaked across the respective agar plates and incubated at 37 ºC for 24 hours. From the incubated plates, inoculums were prepared by making a direct broth suspension of four to five well isolated colonies of *Staphylococcus aureus, Escherichia coli, Cutibacterium acnes with* the same morphological type in freshly prepared 10 ml broths in separate test tubes and incubated in a shaking incubator (Camlab, UK) at 37 ºC for 18 h with 200 rpm. The bacterial suspensions were thereafter adjusted 0.5 McFarland standards (equivalent to 1.5 x10^8 CFU/ml). This was done by diluting the 18 h bacterial cultures 1:100 with respective sterile broths before growing them back in the shaking incubator for approximately 2-3 hours (mid-log phase) to obtain 0.08 to 0.10 OD625 corresponding to 1.5 x 10^8 CFU/ml. The correct density of the turbidity standard was verified using a spectrophotometer with a 1-cm light path and matched cuvette (Star Labs, UK) to determine the absorbance at 625nm. Blank of MHB alone was used to calibrate the spectrophotometer before measuring the samples. To ensure conformity of the suspension's turbidity with McFarland standard, both the suspensions and the prepared McFarland standard were also compared visually. Furthermore, the inoculums suspension was used within 30 minutes of standardization, which is a very important factor to avoid any change of the size of inoculums or loss of their viability.

To investigate the antifungal activity of the African walnut oil, the fungal suspensions used was prepared using three to four morphologically similar colonies of the fungal strains; *Candida albicans* and *Malassezia furfur* from a 24 h culture on Sabouraud dextrose agar. The turbidity of the fungal suspension was adjusted to 1.0 McFarland standard (equivalent to 1.5 × 10^8 CFU/ml) with sterile normal saline (0.89% NaCl wt/vol). All experiments were performed in duplicate and repeated three times.

2.7 Determination of Antimicrobial Activity of African walnut oil

2.7.1 Agar well diffusion method

The antimicrobial activity of different concentrations of the oil was determined by modified agar well diffusion method described by Perez *et al*., (1990) and Adeniyi *et al*., (1996). In this method, nutrient agar and Mueller Hinton agar (MHA) plates were seeded with 0.2 mL of 18 h broth cultures of each bacterial isolate. For the Fungal isolates Sabouraud's Dextrose Agar (SDA) was seeded with 0.2 mL of 24 h broth cultures of *Candida albicans, and Malassezia furfur*. The agar plates, nutrient agar, Mueller Hinton agar (MHA) and Sabouraud's Dextrose Agar (SDA) were separately seeded by spreading a small volume (0.2 mL) of the liquid inoculums (sub-cultured broth media of both bacteria and fungi isolate) by means of an L-shaped glass rod (or a "spreader") in such a way that the surface of the agar in the plates were covered with the microbes (test organisms). All test microorganisms were separately seeded into different plates. All the plates were left to dry for 1 hour. A sterile 6 mm cork-borer was used to cut two wells of equidistance in each of the plates and 0.2 mL (200 µl) of the African walnut oil was introduced into one of the two wells while the same amount of sterile oil was introduced into the second well as control (used as negative control) and all the plates were aerobically incubated at 37 °C for 24 hours for the bacteria and 48 hours for the fungi. The diameter of zones of inhibition was measured by means of linear instrument in millimeter (venier calliper) and recorded.

Antimicrobial activity of African walnut oil diluted in solvents such as petroleum ether and n-hexane was also determined. This was determined by diluting African walnut oil in petroleum ether and n-hexane to get concentrations of (vol/vol) 80, 40, 20, 10 and 5 % of African walnut oil in the solvent. The different concentration of the African walnut oil was incorporated into wells on agar plates and agar plates were incubated as described above. After incubation the diameter of zones of inhibition of each concentration was measured.

2.8 Phytochemical screening of African walnut oil

Qualitative phytochemical constituents of African walnut oil

Qualitative phytochemical analysis of African walnut oil for the presence of flavonoids, alkaloids, saponins, tannins, phenol, terpenoids, glycoside, reducing sugar, hydrogen cyanide and soluble carbohydrate were determined by the standard procedures described by Harborne, (1973) and Mbatchou *et al*., (2011).

Test for Flavonoids

Shinoda's Test

Two ml (2 ml) of the test sample was put in 250 ml beaker and dissolved with 3 ml of ethyl alcohol. Then ten drops (10 drops) of diluted HCL and little magnesium turnings/lead acetate was added. Formation of colours such as reddish, pink, or brown indicated the presence of flavonoids in the sample (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Alkaloids

Wagner's Test

Five hundred microlitre (500 ul) of the test sample was put in a test tube and acidified with 1 ml of 1.5% (v/v) HCL. This solution was dissolved with 500 ul of Wagner's reagent which iodine is prepared in potassium iodide solutions. Within 10 minutes a yellow or brown precipitate of the oil sample were observed. Appearance of yellow or brown precipitate indicated the presence of alkaloids (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Saponins

Foam test

To 2 ml of the test sample, 6 ml of deionized water was added and shaken vigorously; the presence of foams or bubbles indicated the presence of saponins (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Tannins

To 2 ml of the test sample, 500 µl of 10% of alcoholic ferric chloride was added; formation of brownish or blue-black colour indicated the presence of tannins (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Phenol

To 2 ml of the test sample 2 ml of 5% aqueous ferric chloride were added and formation of blue colour within 3 minutes indicated the presence of phenols in the sample (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Terpenoids

To 1 ml of the test sample placed in a 250 ml beaker, 500 µl of 100% chloroform was added followed by addition of 3-5 drops of concentrated sulphuric acid (100 %), formation of dark reddish-brown precipitate of the oil sample indicated the presence of terpenoids (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Glycosides

1ml of concentrated H_2SO_4 was prepared in a test tube. Then 5ml of the test sample was mixed with 2ml of glacial acetic acid (CH_3CO_2H), containing 1drop of $FeCl_3$. The mixture was carefully added to1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture; observation for a brick-red precipitate was made, which indicated the presence of cardiac glycosides (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Reducing Sugar

Five millilitre (5 ml) of a mixture of equal parts of Fehling's solution A and B was added to 5 ml of the sample and then heated in a water bath for 5 min. Brick red precipitate showed the presence of reducing sugar (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Cyanide

Distilled water (15 ml) was added to 0.1 g of the test sample in a test tube. An alkaline picrate paper was suspended over the mixture and held in place by rubber bung. The solution was allowed to stand for 18 hr at room temperature. Colour change from yellow to orange indicated the presence of cyanide (Harborne, 1973; Mbatchou *et al*., 2011).

Test for Soluble Carbohydrate

The test sample (0.1 g) was boiled with 2 ml of distilled water and filtered. To the filtrate, few drops of naphthol solution in ethanol (molisch's reagent) were added. Concentrated sulphuric acid in a Pasteur pipette was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrate (Harborne, 1973; Mbatchou *et al*., 2011).

Quantitative phytochemical constituents of African walnut oil

Quantitative phytochemical screening of African walnut oil for the abundance of flavonoids, alkaloids, saponins, tannins, phenol, terpenoids, glycoside, reducing sugar, hydrogen cyanide and soluble carbohydrate were determined by the standard procedures described by Harborne, (1973), Nyong *et al*., (2009) and Igara *et al*., (2017).

Flavonoids Determination

Ten grams (10 g) of the test sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The resultant solution was then filtered through Whatman filter paper No. 42. The filtrate was then transferred into a crucible and evaporated to dryness over a water bath and weighed to constant weight (Igara *et al*., (2017).

$$\% \text{ Flavonoid} = \frac{\text{Weight of dried sample}}{\text{Weight of sample}} \times 100$$

Alkaloids Determination

Five grams (5 g) of the sample was weighed into 250 ml beaker and 200 ml of 20 % acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and the sample was concentrated to a quarter of the original volume. Then concentrated ammonium hydroxide was added drop by drop to the sample to precipitate the alkaloid. Addition was done continually until the precipitation was complete. The whole solution was allowed to settle and the resultant precipitate was collected by filtration, dried and re-weighed. The percentage alkaloid was calculated as the difference in weight (Harborne, 1973; Nyong *et al*., 2009; Igara *et al*., 2017).

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$\frac{W_1-W_2}{W}$ x 100

W

$-W$

1

W

$x100$

W= weight of the sample

W_1= weight of empty filter paper

W_2= weight of sample + empty filter paper

Saponins Determination

Twenty grams (20 g) of the test sample was dispersed in 200 ml of 20 % ethanol, the suspension was heated on a water bath for 4 hours with continuous stirring at about 55 ^{O} C. The resultant mixture was filtered and residue re-extracted again with 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{O} C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ethanol layer was discarded. The purification process was repeated. Sixty ml (60 ml) of n-butanol was added. The sample was washed twice with 10 ml of 5 % aqueous sodium chloride and the remaining solution was heated in a water bath and allowed to evaporate. After evaporation, the sample was oven-dried to a constant weight and the saponins content was calculated in percentage as difference in weight (Harborne, 1973; Nyong *et al*., 2009).

Tannins Determination

Five hundred milligram of the test sample was weighed into 100 ml plastic bottle and 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask then 5 ml of the filtrate was pipetted out into a tube and mixed with 3 ml of 0.1 M $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferricyanide added. Within 10minutes the absorbance was measured in a spectrophotometer at 120 nm wavelength. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (Harborne, 1973; Nyong *et al*., 2009).

Phenol Content Determination

The quantity of phenol in the test sample was determined using the spectrophotometric method. 2g of the test sample was added to 50ml of ethanoic acid and allowed to boil for 15minutes and left to cool. After cooling 5ml of the boiled sample was pipette into a 50ml conical flask, and 10ml of distilled water was added to it. Also 2ml of NH_4OH and 5ml of concentrated pentanol were added to the mixture and mixed vigorously. After mixing the sample was made up to the reference mark of the flask and allowed for 30minutes for possible colour change; it was then collected and viewed in a U.V. spectrophotometer at 505nm wavelength. The reading represents the phenol content, and was noted accordingly (in mg of solution per 100g of sample) (Harborne, 1973; Nyong *et al*., 2009).

Terpenoid Determination

5ml of the test sample was pipetted into the test tube and put into a sizeable beaker, and 2ml of chloroform was added. Then 0.1ml of H2SO4 (Sulfuric acid) was added. The terpeniods content was read on the calibration curve and noted. The blank solution was prepared using the same procedure. The terpenoids content was determined using the equation

Terpenoids Content, $\frac{mg}{100\ mg}$ = $\frac{\text{Conc. of standard X Absorbance of Sample}}{\text{Absorbance of Standard X Weight of Sample}}$

Glycosides Determination

The test sample (1 g) was mixed with 50 ml of distilled water and filtered. To the filterate (1 ml), 4 ml of alkaline pirate solution was added. The mixture was boiled for 5 min and allowed to cool. The absorbance was read at 490 nm (Harborne, 1973; Nyong *et al*., 2009; Igara *et al*., 2017).

Reducing Sugar Determination

The test sample (1 g) was mixed with 20 ml of distilled water and filtered. To 1 ml of the filterate, 1 ml of alkaline copper reagent was added. The mixture was boiled for 5 min and allowed to cool. Then 1 ml of phosphomolybdic acid reagent and 2 ml of distilled water was added to the sample and the absorbance read at 420 nm (Harborne, 1973; Nyong *et al*., 2009; Igara *et al*., 2017).

Cyanide Determination

The test sample (1 g) was mixed with 50 ml of distilled water and then filtered. To 1 ml of the filterate, 4 ml of alkaline picrate solution was added. The mixture was boiled for 5 min and allowed to cool. The absorbance was measured at 490 nm (Harborne, 1973; Igara *et al*., 2017).

Soluble Carbohydrate Determination

The test sample (1 g) was mixed with 50 ml of distilled water and filtered. To the 1 ml of the filterate, saturated aqueous solution of picric acid was added and absorbance was read at 580 nm (Harborne, 1973; Igara *et al*., 2017).

3. Results

Phytochemical screening of African walnut oil

Qualitative phytochemical results of African walnut oil

The qualitative phytochemical constituents of African walnut oil are shown in Table 2. Analysis showed the presence of flavonoids, alkaloids, saponins, tannins, phenol, glycosides and reducing sugar in African walnut oil. The result revealed the high presence and abundance of alkaloids, saponins, phenol, glycosides and reducing sugar in the nut oil. Moderate amount of flavonoid was observed in the oil. However, only tannins and soluble carbohydrate was observed in trace amount while terpenoids and hydrogen cyanide were absent or not detected in the oil sample.

Table 2: Qualitative phytochemical screening results of Africa walnut oil

Phytochemical constituents	Qualitative analysis result
Flavonoids	+ +
Alkaloids	+ + +
Saponins	+ + +
Tannins	+
Phenol	+ + +
Terpenoids	ND
Glycosides	+ + +
Reducing Sugar	+ + +
Soluble carbohydrate	+
Hydrogen cyanide	ND

Key:
+ = present and available in minute/trace amount.
++ = present and available in moderate amount
+++ = present and available in high and appreciable amount
ND = not detected/ absent

Quantitative phytochemical results of African walnut oil

Table 3 shows the phytochemical constituents present in African walnut oil. Results in table 3 shows the constituents present in African walnut oil analysed. The results obtained show high concentration of alkaloids (3.87 ± 0.02 mg/100 g), saponins (4.37 ± 0.01 mg/100 g), phenol (2.32 ± 0.02 mg/100 g), glycoside (2.66 ± 0.02 mg/100 g), reducing Sugar (3.04 ± 0.02 mg/100 g), moderate concentration of flavonoids (1.98 ± 0.02 mg/100 g), minute or trace amount of tannins (0.85 ± 0.02 mg/100 g) and soluble carbohydrate (0.99 ± 0.01 mg/100 g) while terpenoids and hydrogen cyanide were not detected in the oil sample.

Table 3: Quantitative phytochemical screening results of Africa walnut oil

Phytochemical constituents	Mean composition (mg/100 g dry walnut weight)
Flavonoids (mg/100 g)	1.98 ± 0.02
Alkaloids (mg/100 g)	3.87 ± 0.02
Saponins (mg/100 g)	4.37 ± 0.01
Tannins (mg/100 g)	0.85 ± 0.02
Phenol (mg/100 g)	2.32 ± 0.02
Terpenoids (mg/100 g)	ND
Glycoside (mg/100 g)	2.66 ± 0.02
Reducing Sugar (mg/100 g)	3.04 ± 0.02
Soluble carbohydrate (mg/100 g)	0.99 ± 0.01
Hydrogen Cyanide (mg/100 g)	ND

Values are means of triplicate readings ± SD

Microbiological screening

Antimicrobial activity of African walnut oil

The result of the antibacterial and antifungal activity of African walnut oil is presented in Table 4. The result of this investigation showed that African walnut oil exhibited inhibitory effect on the growth of clinical isolates of *Staphylococcus aureus, Escherichia coli, Cutibacterium acnes, Malassezia furfur* and *Candida albicans* tested. Significant differences (p<0.05) were observed in the degree of inhibition of the clinical isolates, but non-significant variations were observed inhibition among strains of the same species. On the bacterial species, *S. aureus* was highly inhibited by African walnut oil as confirmed by the zones of inhibition measured. *C. acnes* was moderately inhibited, while the growth of *E. coli* was observed to mildly inhibited. With the fungal species, 100 % of African walnut oil proved really effective inhibiting the growth of *M. furfur* and *C. albicans.* However, growth inhibition by African walnut oil was not significant between the two strains.

The result obtained also revealed that the inhibitory activity of the nut oil decreases with a decrease in concentration of oil in solvent, resulting in marked variation in the inhibitory concentration. While 5 % of African walnut oil was still able to inhibit the growth of *S. aureus*, inhibition of *E. coli* was only achieved at a much higher concentration of 80 % oil in solvent. *C. acnes* and *M. furfur* were inhibited at 20 % and *C. albicans* was inhibited at 10 % concentration.

The type of solvent used for the dilution of African walnut oil did not seem to have any significant (p<0.05) effect on growth inhibition of all the bacterial isolates tested, however for the fungi species inhibitory was at a lower concentration with oil diluted in n-hexane. As observed, a concentration of 10 % for *M. furfur* and 5 % for *C albicans* with petroleum ether compared with 20 % and 10 % respectively for *M. furfur* and *C albicans* with n-hexane as the solvent.

Table 4: Inhibitory effect of 100 % African walnut oil and different concentration of nut oil on growth of test microorganisms

			Diameter of zones of inhibition (in mm)											
			Petroleum ether						n-hexane					
			Concentration (v/v)						Concentration (v/v)					
Microorganisms	Strain	100% African walnut oil	80	40	20	10	5	Control	80	40	20	10	5	Control
Staphylococcus aureus	2341	14.02	12.09	10.09	6.03	4.19	2.66	0.00	10.28	8.74	4.77	4.35	3.05	0.00
	3411	13.21	11.07	10.05	6.91	4.07	2.08	0.00	10.76	9.56	6.78	4.89	2.17	0.00
	3621	12.35	12.12	11.06	8.56	5.33	3.76	0.00	10.14	9.21	5.67	4.32	3.56	0.00
	3001	13.32	12.45	11.04	8.17	4.87	3.32	0.00	10.99	9.35	5.99	4.44	3.33	0.00
	1990	13.65	11.22	11.43	6.17	4.34	3.87	0.00	10.94	8.89	6.34	4.07	3.05	0.00
Escherichia coli	2333	2.89	1.87	0.76	0.00	0.00	0.00	0.00	0.87	0.00	0.00	0.00	0.00	0.00
	1411	1.87	1.09	0.88	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00
	1093	1.56	1.08	0.53	0.00	0.00	0.00	0.00	0.49	0.00	0.00	0.00	0.00	0.00
	1114	1.48	1.43	0.65	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00
	1039	1.85	1.49	0.67	0.00	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00
Cutibacterium acnes	3143	5.54	4.88	4.09	2.23	0.00	0.00	0.00	3.09	2.33	1.98	0.00	0.00	0.00
	2030	4.87	4.88	3.56	2.45	0.00	0.00	0.00	1.98	1.09	0.56	0.00	0.00	0.00
	1190	3.76	3.76	3.23	1.06	0.00	0.00	0.00	1.45	0.56	0.46	0.00	0.00	0.00
	1390	5.78	4.56	3.12	1.04	0.00	0.00	0.00	1.06	0.75	0.58	0.00	0.00	0.00
	1102	4.21	3.34	3.25	1.09	0.00	0.00	0.00	1.09	0.67	0.55	0.00	0.00	0.00
Malassezia furfur	2556	9.65	6.73	3.62	1.99	1.08	0.00	0.00	7.06	4.32	2.33	0.00	0.00	0.00
	2035	9.43	6.43	4.23	1.45	1.21	0.00	0.00	7.25	4.44	2.14	0.00	0.00	0.00
	2441	8.77	6.83	5.55	1.22	1.06	0.00	0.00	7.21	3.11	1.89	0.00	0.00	0.00
	2099	8.43	5.39	4.56	1.33	1.11	0.00	0.00	6.52	3.08	1.22	0.00	0.00	0.00
	2016	7.88	5.09	3.21	1.01	1.23	0.00	0.00	6.23	2.13	1.48	0.00	0.00	0.00
Candida albicans	1001	9.98	8.04	8.01	6.21	4.09	1.02	0.00	8.21	6.32	5.09	4.44	1.26	0.00
	1425	9.36	8.21	8.34	5.34	3.23	1.04	0.00	8.31	6.23	5.21	4.25	1.35	0.00
	1346	9.41	8.33	7.34	5.25	3.06	0.00	0.00	7.09	6.09	5.01	4.31	1.37	0.00
	1922	9.22	7.45	6.9	4.33	3.01	0.00	0.00	6.03	7.33	5.11	4.22	1.05	0.00
	2140	8.01	7.22	6.45	4.21	3.21	0.00	0.00	6.02	7.21	5.21	4.09	1.11	0.00

4. **Discussion**

The qualitative phytochemical screening of African walnut oil revealed the presence of phytochemical components such as flavonoids, alkaloids, saponins, tannins, glycosides, phenol and reducing sugar. The nut oil significantly showed higher presence and appreciable amount of alkaloids, saponins, phenol, glycosides and reducing sugar and moderate amount of flavonoids. Trace amount of tannins and soluble carbohydrate was observed while terpenoid and hydrogen cyanide were absent. To date, this is the first study to give detailed phytochemical composition of African walnut oil in Nigeria. However, phytochemical constituents found in African walnut oil are compared to those reported by other researchers in literature for parts of African walnut plant. Our report on phytochemicals present in African walnut oil is similar to that of Grace *et al*., (2016) who reported the presence and appreciable amount of alkaloids, glycosides and reducing sugar in the hull and nut of African walnut. This result is in conformity with that of Iyewoka and co-workers (2016) who reported that the root bark of African walnut plant contained alkaloids and glycosides in appreciable amount too. Also, the result of the phytochemical constituents of the seed of African walnut reported by Nyong *et al*., (2009), showed that alkaloids, tannins, flavonoids, saponnins and glycosides are present in appreciable amount and hydrogen cyanide and terpenoids were not detected. Furthermore, the results obtained in this study does not seem to correlate with those reported by Nwaoguikpe *et al*.,(2018) in differently processed African walnut. In Nwaoguikpe *et al*.,(2018) study, only saponin was available in appreciable amount, flavonoid was present in moderate amount while tannin and alkaloids were observed in trace amount. However, the result differs slightly to those of Onawunmi *et al*., (2013) who reported the moderate presence of tannin, saponnin and alkaloids in African walnut leaf. The high and appreciable amounts of alkaloids, saponins, glycosides and reducing sugar observed in African walnut oil compares favorably well with the findings of Chijioke *et al*.,(2015). Lastly, the presence of phenol in African walnut oil corroborate with the report of Igara *et al*., (2017) who reported appreciable amount of phenol in African walnut.

Phytochemicals are substances produced by plants, and these substances have biological activity (Mendoza and Silva, 2018). Plants represent the main source to obtain various active ingredients. They exhibit pharmacological effects applicable to the treatment of numerous microbial infections (Mendoza and Silva, 2018).The phytochemical screening of African walnut oil in this study showed that the oil contain appreciable amount of phytochemicals and also possess antibacterial properties. African walnut oil also contains high concentration and appreciable amount of alkaloids. Alkaloids contain nitrogen bases and have important effects on humans. It has been linked with medicinal uses for centuries. Most common biological properties of alkaloids are their toxicity against cells of foreign organisms, anti-inflammatory, anti-asthmatic and anti-allergic properties. Alkaloids are used in reducing body pains, anxiety and depression (Wintola and Afolayan, 2015). The presence of alkaloids in the oil of African walnut suggests that antimicrobial activities of African walnut oil may be due to the alkaloids. However, the presence of saponins in high concentration in African walnut oil is in agreement with the report of the earlier report of other researchers (Ayoola *et al*., 2011; Onawunmi *et al*., 2013; Chijioke *et al*., 2015) for walnut root, seeds and leaf. Result obtained is an indication that walnut has cytotoxic effect on the intestine. Saponin gives the walnut plant its bitter taste (Chijioke *et al*., 2015). The presence of tannins and saponins in African walnut oil suggest that it could be used for healing of varicose ulcers, haemorrhoids and treatment of bacterial and fungal infections (Nyong *et al*., 2009). Apart from alkaloids, the presence of flavonoids in the oil of African walnut is important since they have been reported to exhibit antimicrobial, anti-inflammatory, analgesic, anti-allergic, antioxidant, anticancer and antitrypanosomal activity (Ekam and Ebong, 2007; Nyong *et al*., 2009; Wintola and Afolayan, 2015). They are sometimes used for therapeutic purposes (Nyong *et al*., 2009). The appreciable amount of flavonoids content observed in African walnut oil account for the wide range of biological activities reported about it which is obvious in the antimicrobial activity of the plant (Nyong *et al*., 2009).

The antimicrobial activity of African walnut oil as observed in this study appeared to be a broad spectrum activity as both gram positive, gram negative as well as fungal species were sensitive to the nut oil. The test microorganisms chosen for this study are known primarily as commensals or normal flora on the human skin but they can be opportunistic as they have the ability to change from a commensal or normal flora to pathogenic strains when the environment, that is the human skin supports their growth and especially when the immune system of the host is lowered or compromised. The implication of the effect of nut oil on growth inhibition is that the nut oil can help keep the pathogenic activities of these microrganisms in check. *Staphylococcus aureus* which is commonly found associated with boil and other secondary skin infections in the area of study was most inhibited. The inhibition of *E.coli* by the oil was relatively low, while that of *C.acnes* was mild compared to *S. aureus.* Previous research studies on the antimicrobial activities of plant oil component indicated that lemon, cineole, citral geraniol, linalool and menthol were active against several yeast –like and filamentous fungus (Pattnaik *et al*., 1997). Previous reports have also shown that essential oils of plant sources could be used as therapeutic agent for the remedy of fungal diseases of man (Tampieri *et al*., 2005) and plant (Soylu *et al*., 2006).

5. Conclusions

African walnut oil demonstrated a broad spectrum activity on bacterial and fungal clinical isolates tested. The phytochemicals present in African walnut oil no doubt contributed to its activity against bacterial and fungal skin pathogens causing skin infections in human and thus justifies its continued use in traditional medicine.

References

Adeniyi, B. A., Odelola, H. A., and Oso, B. A. (1996). Antimicrobial potentials of *Diospyros mespiliformis* (Ebenaceae). *African Journal of Medicine and Medical Sciences*, *25*(3), 221-224.

Ajaiyeoba, E. O., and Fadare, D. A. (2006). Antimicrobial potential of extracts and fractions of the African walnut–*Tetracarpidium conophorum*. *African Journal of Biotechnology*, *5*(22).

Ayodeji, A. E., and Aliyu, N. (2018). *Tetracarpidium conophorum* (African walnut) Hutch. & Dalziel: Ethnomedicinal uses and its therapeutic activities. *Journal of Medicinal Plants for Economic Development*, *2*(1), 1-10.

Ayoola, P. B.; Adeyeye, A.; Onawumi, O. O. and Faboya, O. O. P. (2011). Phytochemical and Nutrient Evaluation of *Tetracarpidium Conophorum* (Nigerian Walnut) Root, IJRRAS., 7(2): 197 – 202, 2011.

Bello, O. S., Olaifa, F. E., Emikpe, B. O., and Ogunbanwo, S. T. (2013). Potentials of walnut (*tetracarpidium conophorum Mull. Arg*) leaf and onions (*allium cepa linn*) bulb extracts as antimicrobial agents for fish. *African Journal of Microbiological Research*, *7*(19), 2028-2033

Cheesbrough, M. District Laboratory Practice in Tropical Countries, Cambridge University Press, UK. 2(2), 2010, 143-240.

Chijioke, O. C., Anosike Chioma, A., and Collins, A. C. (2015). Studies on the phytochemical and nutritional properties of *Tetracarpidium conophorum* (black walnut) seeds. *Journal of Global Bioscience*, *4*(2), 1366-1372.

Edem, C. A., Dosunmu, M. I., and Bassey, F. I. (2009). Determination of proximate composition, ascorbic acid and heavy metal content of African walnut (*Tetracarpidium conophorum*). *Pakistan Journal of Nutrition*, *8*(3), 225-226.

Ekwe, C. C., and Ihemeje, A. (2013). Evaluation of physiochemical properties and preservation of African walnut (*Tetracarpidium conophorum*). *Academic Research International*, *4*(6), 501.

Harbone J.B. (1973). Phytochemical Methods. Chapman and Hall, London. Pp: 110-113.

Igara, C. E., Omoboyowa, D. A., Uchegbu, R. I., and Ahuchaogu, A. A. (2017). Phytochemical composition, proximate analysis and vitamin content of (*Tetracarpidium conophorum*) wall nut seed. *Chem. Res. J*, *2*(5), 1-8.

Irobi, O. N., and Daramola, S. O. (1993). Antifungal activities of crude extracts of *Mitracarpus villosus* (Rubiaceae). *Journal of ethnopharmacology*, *40*(2), 137-140.

Iyekowa, O., Owolabi, B. J., and Cole, O. (2016). Gas Chromatography (GC)–Mass Spectrometry (MS) analysis of the isolated oil of *Tetracarpidium conophorum* (wallnut). Mull (Arg) root bark. Scientia Africana, 15(1).

Lamichhane, S., Hussain, A., Paudel, M., and Jha, R. N. (2016). Phytochemical, antimicrobial and GC-MS analysis of *Junglans regia* Linn. *Chem. Sci. Rev. Lett*, *5*(20), 147-156.

Mamedov, N., Gardner, Z., and Craker, L. E. (2005). Medicinal plants used in Russia and Central Asia for the treatment of selected skin conditions. *Journal of herbs, spices & medicinal plants*, *11*(1-2), 191-222.

Mendoza, N., and Silva, E. M. E. (2018). Introduction to phytochemicals: secondary metabolites from plants with active principles for pharmacological importance. *Phytochemicals: Source of Antioxidants and Role in Disease Prevention*, 25.

Nwaichi, E.O., Osuoha, J.O. and Monanu, M.O. (2017), 'Nutraceutical potential of *Tetracarpidium conophorum* and *Buccholzia coriacea* in diet-induced hyperlipidemia', *Journal of Chemical Health Risks* 7(3), 157–170.

Nwaoguikpe, R. N., Ujowundu, C. O., and Wesley, B. (2012). Phytochemical and biochemical compositions of African walnut (*Tetracarpidium conophorum*). *Journal of Pharmaceutical and Biomedical Sciences©(JPBMS)*, *20*(20).

Nyong, B. E., Edem, C. A., Obochi, G. O., and Malu, S. P. (2009). Effect of methods of extraction on phytochemical constituents and antibacterial properties of *Tetracarpidium conophorum* seeds. Global journal of pure and applied sciences, 15(3-4).

Obianime, A. W. and Uche, F. I. (2010). The effects of aqueous extracts of *Tetracarpidium conophorum* seeds on the hormonal parameters of male guinea pigs, *Asia Pacific Journal of Tropical Medicine* 3(1), 21–24.

Ogunwusi, A. A., and Ibrahim, H. D. (2016). Industrial potential of underutilized plants in Nigeria. *Journal Natural Science Research*, *6*, 20-28.

Okeke, M. I., Iroegbu, C. U., Jideofor, C. O., Okoli, A. S., and Esimone, C. O. (2001). Anti-microbial activity of ethanol extracts of two indigenous Nigerian spices. *Journal of herbs, spices & medicinal plants*, *8*(4), 39-46.

Onawumi, O. O. E., Faboya, O. O. P., and Ayoola, P. B. (2013). Chemical evaluation and nutritive values of African walnut leaf (Plukenetia conophora Mull. arg.). *International Journal of Herbal Medicine*, *1*(3), 122-126.

Pattnaik, S., Subramanyam, V. R., Bapaji, M., and Kole, C. R. (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios*, *89*(358), 39-46.

Pawar, V. C., and Thaker, V. S. (2006). In vitro efficacy of 75 essential oils against *Aspergillus niger*. *Mycoses*, *49*(4), 316-323.

Perez-Eid, C., Pauli, W. M., & Bazerque, P. (1990). An antibiotic assay by agar-well diffusion method. Acta Biol. Med. Exp.15:113-115

Raja, G., Shaker, I. A., Sailaja, I., Swaminathan, R., Babu, K. S., & Basha, S. S. (2012). Nutritional analysis of nuts extract of *Juglans regia* l. *International Journal of Bioassays*, *1*(10), 68-73.

Sen, A., & Batra, A. (2012). Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. *Int J Curr Pharm Res*, *4*(2), 67-73.

Soylu, E. M., Soylu, S., & Kurt, S. (2006). Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent Phytophthora infestans. *Mycopathologia*, *161*(2), 119-128.

Tampieri, M. P., Galuppi, R., Macchioni, F., Carelle, M. S., Falcioni, L., Cioni, P. L., & Morelli, I. (2005). The inhibition of Candida albicans by selected essential oils and their major components. *Mycopathologia*, *159*(3), 339-345.

Udedi, S.C.A., Ani, O.N.A., Anajekwu, B.N.A., Ononamadu, C.J.A., Igwilo, I.O.A., Ibeabuchi, C.G.A. et al., 2014, 'Nutritional composition and antioxidant activity of African walnut, (*Tetracarpidium conophorum*)', *The Journal of Applied Biochemistry* 107, 170–180.

Wintola, O. A., & Afolayan, A. J. (2015). The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of Hydnora africana Thunb. used as antidysenteric in Eastern Cape Province, South Africa. *BMC Complementary and Alternative Medicine*, *15*(1), 307.