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Phytochemical And Antimicrobial Activities of Seed Ethanolic Extract of African Walnut on Selected Pathogens

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ABSTRACT

African walnuts is a member of the euphorbiaceae the genus plukentia. Phytochemical and antimicrobial activities of African walnut (Teracarpidium conophorum) ethanolic extract were investigated. The effect of this seed was examined on 3 selected groups of microorganisms. Escherichia coliwere selected among gram negative bacteria. Staphylococcus aureus among gram positive bacteria. While among group of fungi Candida albican was selected. The extraction of active substance was done by soaking the dried powered seed in ethanol for 72hours and it was evaporated using water bath at required temperature. Disc diffusion method was used for examination of antimicrobial activities of the extract. Result shows that the extract has little effect on Escherichia coli, it has no effect on Staphylococcus aureusas well as Candida albican. Phytochemical Constituents presence were alkaloids, flavonoids, terpenoids, tannis, sapannin, glycosides and anthiaquinones. The presence of these phytochemical constituents supports its uses in herbal medicine as therapeutic agent. More research has to be done on the importance of oil fraction of this seed plant.

INTRODUCTION

The African walnut (*Tetracarpidium conophorum*) is a member of the euphorbiaceae family and the genus plukentia (GRIN, 2020). It is also called Nigerian walnut. Black walnut (*Tetracarpidium conophorum*) is a perennial climbing shrub. 100m-200m long, often found in most forests being so named because its nuts bear a superficial resemblance to the walnut. An African walnut is native to tropical western and central Africa, from Togo to Congo and Sierra Leone and is abundant in Nigeria, Cameroon, Republic of the Congo and theDemocratic Republic of Congo. It prefers rain forest hedge in half-shady places, low bush,secondary forest, plantain at elevations from 250-1400m and produced sterns usually 3-15cm long though they can be up to 30m long (Chikezie, 2019). *Tetracarpidium conophorum*is known in southern Nigeria as Ukpa (Igbo), in Western Nigeria as Awusa or Asala (Yoruba) and is known as KasoorNgak in the literal and western Cameroon (Malu et al., 2019). It is known in northern Nigeria as Gawudibairi (Hausa). The plant is found ¡n Lagos. Uyo, Akamkpa. Akpabuyo, Kogi, Ogbomoso and Ibadan (Ayoola et al., 2021)

The plant is cultivated principally for the nuts which are cooked and consumed as snacks and the nut is an excellent source of protein and provide high food energy value (Nwaoguikpe et al., 2022) amid (Ojobor el at., 2018). The nut is a thin-shelled nut and about 25mm long. It is contained in a pod which may have one, two or three-shelled nut. The walnut shells could he black or brown from the plant. The nut is whitish upon cracking from the shell. The nut has adim layer in between two halves (Ayoola et al., 2021). The seeds takes 4 - 6 months to mature and are found in the local market between the months of June and September in Nigeria. It is traditionally eaten as nut after boiling (Akpuaka and Nwankwo, 2020).

African walnuts are edible even when raw and give a bitter taste and a stimulating effect like kola. They can be cooked, roasted or sun dried and the roasted seeds could be ground like melon seeds and used as a thickener in soup preparation. The plant is known in Africa especially in the eastern and western parts of Nigeria for its antibacterial efficacy (Okerulu and Ani, 2021). Decoction of leaves and seeds serve as beverages which relieves abdominal pain and fever (Malu et al, 2019). Dried walnuts can be ground and turned into flour which j can be used as composite flour during baking or in place of milk in tea preparation (Ekwe and Ihemeje, 2023). The African walnut seed can also be used in the treatment of dysentery andfibroid (NNMDA, 2018).

Balch and Balch (2020) stated that walnut can be fully benefitted from when consumed freshin their raw form, the reason is that up to 15% of it healthy oils and phytochemicalconstituents that naturally occur in walnut are lost during processing. In the southern Nigeria ethano-medicine, the eaves back and fruits of the conopher plant are considered effective in curing toothache, syphilis and as an antidote to snake bite (Ekwe and Iherneje, 2023). The aim of this work is to determine the Phytochemical and antimicrobial activities of seed ethanolic extract of African Walnut (*Tetracarpidium conophorum*) on selected pathogen.

Materials and Methods

Study Area

The research study was carried out in the microbiology laboratory of the Federal Polytechnic Bida, Niger State, North Central, Nigeria.

Materials

Analytical weighing balance, incubator, autoclave, water bath, fridge, petri dishes, test tubes, Bunsen burner, beaker, electronic blender, measuring cylinder, conical flasks. whatmanfilter paper, inoculating loop, muslin cloth, reagent screw cap bottle, cotton wool, aluminum foilpaper, Nutrient Agar (NA), Sabouraud's Dextrose Agar (SDA), Ethanol, distilled water, iron (vi) chloride, Foric chloride, concentrated hydrogen tetrasulphate (vi) acid, felying solution, and tetrasulphate (iii) acid, *Staphylococcus aureus, Escherichiehracoli, Candida albieans.*

Collection of Sample

The African walnut (Tetracarpidium conophorum) seeds were obtained at odo-ori market, Iwo, Osmi state, Nigeria and it was transported to microbiology laboratory of the FederalPolytechnic Bida, Niger state, Nigeria. The shell of the seeds was removed and they were cut into small pieces. This was done to enable the seeds to dry.

The seeds were then sun-dried until they were completely dried off. The dried seeds were pounded with sterile mortar and pestle before blending with sterile electronic blender into fine powder (Ajaiyeoba, 2022).

Extraction of Active Substances of African Walnut Seeds

The extraction was done using the method described by Ajaiyeoba (2022). 250g of the powered seeds were weighed into l000ml of solvent (ethanol) in a sterile screwed cap bottle and it was stored for 72 hours for adequate extraction at room temperature with an intermittent agitation. The mixture was then sieved first with muslin cloth and then through whatman filter paper. The filtrate was then evaporated in a water bath, reflux at low pressure until it is in paste form. The concentrated extract was then stored in a refrigerator at 40°c ¡n a screw cap bottle.

Phytochemical Screening

The phytochemical screening was done using the methods described by Chikezie, (2019) to determine saponins, tannins, phenols, sterriods and glycosides. Saponnins was determined by vigorously shaking 2mnls of the aqueous extract of the samples for 2 minutes and checked forthe formation of stable foams. The tannins in the sample were detected by mixing 2mls of the aqueous extract with 4mls of 5% iron (iii) chloride and checked for the formation of dark green precipitate. The phenols content present in the seeds extract was determined by adding 2m1 of seeds extract with few drops of ferric chloride solution and checked for either bluish green or red colouration in the mixture. The presence of steroids were determined by adding5 drops of concentrated hydrogen sulphate (H_2S0_4) to 1 ml of seeds extract and checked forthe formation of red colouration. The glycosides test was done by adding 20mls of 50% tetrasulphate (vi) (SO_4) to 2mls of seeds extracts in a test tube, the mixture was then heated in a water bath for few miinutes and it was allowed to boil. Theglycosides were determined with the presence of brick red precipitation.

Antimicrobial Activity (Sensitivity Test)

The disc diffusion method was used as described by Nounagnon et al., (2019) to screen theantimicrobial activity. In vitro antimicrobial assay was done using nutrient agar and Sabouraud's Dextrose Agar for bacteria and fungi respectively. The dishes were prepared by pouring approximately l8mls-20mls of molten media into a sterile petri dish. The organisms suspension that have been adjusted to Mcfarland standard number 1.0 xl06Cfu/ml in a molten nutrient agar and Sabouraud's Dextrose Agar broth respectively and they were inoculated on

previously prepared nutrient agar and sabouraud's dextrose agar plates using streak plate techniques.

Dried and sterilized paper disc of 6mm diameter were treated with 0.5rn1 of previously prepared *Tetracarpidum conophorum* extract solution of different concentration using a micropipette under aseptic condition. The discs were placed at equidistance in a circle on the inoculated plates. These plates were kept for about 15 minutes to 30 minutes at low temperature so as to allow the diffuse from disc to the surrounding media (nutrient and Sabouraud's Dextrose Agar). The plates were then incubated at 37°c for 24 hours and 72 hours respectively. The zone of inhibition was then examined after incubation and the negative control was impregnated with only the extract.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of African walnut seeds extract was performed by macrodilution method described by Nounagnan et al., (2019). 1 ml of sterile broth media Nutrient and Sabouraud's Dextrose were pipette into 6 different sterile test tubes and lml of African walnut seeds (*Tetracarpidium conophorum*) extract was pipette into the first tube then from first lube double dilution was performed by transferring 1mI from previous tube to the next tube up to the 5th tube. All the tubes were inoculated with 1ml of standardized organisms and it was thoroughly vertex. All the tubes were then incubated for 24 hours and 72 hours respectively.After incubating all the tubes were examined for turbidity.

Minimum Bacteriocidal Concentration (MBC)

The MBC was determined by sub-culturing the test dilution of no growth and the last tube with a concentration next to no growth onto a freshly prepared solid medium and then incubated further for 18 - 24 hours. The highest dilution that yields no growth on solid medium was taken as MBC (Saha and Rahman, 2018).

RESULT AND DISCUSSION

The phytochemical constituents of the seed ethanolic extract of African Walnut.

The table below shows the phytochemical constituents that were presence in the seed ethanolic extract of African Walnut (*Tetracarpidium canopharum*).

Table 1: Phytochemical constituents of seed ethanolic extract of African Walnut.

Phytochemical constituent	Result
Sterols	-
Terpenoids	+
Flavonoids	+
Tannins	+
Phlabatannis	-
Glycosides	+

Saponins	+
Anthraguinones	+
Alkaloids	+

+ represent presence of constituent

- represent absence of constituent

Antimicrobial Analysis of Seed Ethanolic Extract of African Walnut Seed

Ethanol Extract

Antimicrobial Analysis of seed ethanolic extract of African Walnut (*Tetracarpidium canopharum*) on *Escherichia coli*.

Table below shows the antimicrobial activities of seed ethanolic extract of African Walnut on *Escherichia coli*

Table 2: Antimicrobial activities of seed Ethaanolic extract of African Walnut on *Escherichia coli*.

Concentration (mg/ml)	Zone of inhibition (mm)
250	11
100	06
50	-

Antimicrobial analysis of seed ethanolic extract of African Walnut (*Tetracarpidiumcanopharum*) on Staphylococcus aureus

Table below shows that seed ethanolic extract of African Walnut was not susceptible to antimicrobial activities of *Staphylococcus aureus*.

Antimicrobial activities of seed ethanolic extract of African Walnut on *Staphylococcus aureus*

Concentration (mg/ml)	Zone of inhibition (mm)
250	-
100	-
50	-

Antimicrobial analysis of seed ethanolic extract of African Walnut on *Candida albican*

The result below shows that seed ethanolic extract of African Walnut has no effect on the activities of *Candida albican.*

Table 4: Antimicrobial activities of seed ethanolic extract of African Walnut on *Candida albican*

Concentration (mg/ml)	Zone of inhibition (mm)
250	-
100	-
50	-

- represent no zone of inhibition

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of seed ethanolic extract of African Walnut was able to inhibit the growth of test organism (*Escherichia coli*).

In the table below the two less concentration of seed ethanolic extract of African Walnut was able to inhibit the growth of test organisms (*Escherichia coli*).

Table 5: Minimum inhibitory concentration of seed ethanolic extract of African Walnut on *Escherichia coli*

Concentration (mg/ml)	**Zone of inhibition (mm)**
250	Turbid
125	Turbid
62.5	Turbid
31.25	Not turbid
15.62	Not turbid

Minimum inhibitory concentration of seed ethanolic extract of African Walnut on *Staphvlococcus aureus*

In the table below the less concentration of seed ethanolic extract of African Walnut was able to inhibit the growth of *Staphylococcus aureus.*

Table 6: Minimum inhibitory concentration of seed ethanolic extract of African Walnut on *Staphylococcus aureus*

Concentration (mg/ml)	**Zone of inhibition (mm)**
250	Turbid
125	Turbid
62.5	Turbid
31.25	Turbid
15.62	Not turbid

Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration of seed ethanolic extract of African Walnut on *Escherichia coli*

In the table below, the o less concentration that were subculture from MIC in two different plates were observed to be no growth on each of the plates.

Table 7: Minimum bactericidal concentration of seed ethanòlic extract of African Walnut on *Escherichia coli*

Plates	Observation
31.25	No growth
15.62	No growth

Minimum bactericidal concentration of seed ethanolic extract of African Walnut on *Staphylococcus aureus*.

Table below shows that the concentrations subculture from MIC were observed to growth on MBC plates

Table 8: Minimum bactericidalconcentration of seed ethanolic extract of African Walnut on *Staphylococcus aureus*

Plates	Observation
31.25	Growth
15.62	Growth

Discussion

Table 1 shows the phytochemical screening results of seed ethanolic extract of African Walnut (*Tetracarpidium conophorum*). It shows that the seeds contain terpenoids, flavonoid, tannins, glycosides, saponins, anthraquinones, and alkaloids. The presence of sapon in shows that *Tetracarpidium conophorum* has cytotoxic effect such as permealization of the intestine, saponin has relationship with sex hormones like oxytocin which is hormone involved in controlling the onset of labour in women and subsequent release of milk, it also found to gives bitter taste upon drinking of water immediately after eating (Okwu and Okwu, 2021). More also alkaloids are the most efficient substances used therapeutically. Pure isolated alkaloids and the synthetic derivatives are used the basic medicinal agents (Ayoola et al., 2021), The presence of tannins in the seed of African walnut can support its strong use for healing of haemorrhoids frost bite and varicose ulcers in herbal medicine (Ayoola e al.,2021). The presence of these phytochemical such as alkaloids,

tannins, terpenoids,flavonoids, glycosides, saponins, anthraquinones partly shows the use of African walnut inherbal medicine.

The result corresponds with the work of Ajayiyeoba and Fadare (2022) and also with result obtained by Ayoola et al., (2021) on phytochemical screening of African walnut root. However the ethanolic extract of African walnut has a little effect on *Escherichia coli*. The result obtained in table 2 shows that the concentration of 250mg/1ml and l00mg/ml were able to produce zone of inhibition of 11mm and 6mm respectively. This shows that the extract was sensitive to *Escherichia coli*.

The edible African walnut seed was devoid of antimicrobial property on *Staphylococcus aureus* and *Candida albician*. The table 3 and 4 shows that the ethanolic extract of African walnut seed do not have any effect on both micro-organisms. All the concentrations used on both *Staphylococcus aureus*. And *Candida albician* were unable to shows any Zone of inhibition. This shows that the extract was not sensitive for *Staphylococcus aureus*. And *Candida albican*.

Table 5 and 6 shows the minimum inhibitory concentration of the Africa walnut seed ethanol extract (*Tetracarpidium canophorum*) on both *Escherichia coli* and *Staphylococcus aureus*respectively. Tubes 4 and 5 with concentration of 31.25mg/ml and 15.62mg/ml were able to inhibit the growth of *Escherichia coli* in which produce clear zone of inhibition after incubation unlike other tubes.

However, only tube 5 with concentration of 15.62mg/ml was able to produce clear zone of inhibitionwhich show; that the concentration inhibits the growthof the organisms. Minimum bactericidal concentration is to determine which concentration was able to eliminate the organism totally from the tubes of inhibition of minimum inhibitory concentration.

Table 7 and 8 shows the minimum bactericidal concentration of *Escherichia coli* and *Staphylococcus aureus* respectively Result obtained shows that the two concentrations thatinhibit the growth of *Escherichia coli* were able to eliminate the organism during the minimum inhibitory concentration examination. Whereas the concentration that inhibits the growth of *Staphylococcus aureus* was not able to eliminate the tested organism but only inhibits its growth. This made it not to produce any zone of inhibition during the Antimicrobial activities examination (sensitivity test). While the ability of the extract to eliminate the *Escherichia coli* made it to produce zone of inhibition.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The present study has shown the phytochemical properties and antimicrobial activities of seed ethanolic extract of African Walnut (*Tetreacarpidium conophorum*). This partly shows the use of this seed in herbal machine due to its source of food and drugs.

The presence of sapnins supports its cytotocic effect and also has relationship in sex hormone, the tannins also support its healing properties and the alkaloids support its uses as therapeutic agents (Chikekie, 2019; Ajaiyeoba and Fadare, 2022).

The antimicrobial activities show that extract has little effects on *Escherichia coli* while it does not have effects on *Staphylococcus aureus* and *Candida albican*.

Recommendations

i. Dues to the phytochemical properties of the seeds, it is therefore recommended that the seeds should be included in the formulation of drugs.
ii. It is also recommended that the seeds should be included in our daily diet.
iii. More research should be done on the important of oil fraction of the seed.
iv. It is also recommended that food industries should include the seed as addictive in their product.
v. More study should be done based on antimicrobial effect on other pathogenic micro-organisms.
vi. Base on unavailability of the plant in some area of the country, people are then recommended on the cultivation of plant in area where it is not cultivated.
vii. It is also recommended that more research should be done on another extraction of seeds and other parts of the plant using other solvents such as acetone, chloroform, hexane.

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