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Phytochemical screening and antimicrobial studies of *afzelia africana* and *detarium microcarpum* seeds

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A B S T R A C T

The aim of this study was to probe the phytochemical constituents and the antimicrobial activities of *Afzelia africana* and *Detarium microcarpum* seed endosperms. The results obtained from the phytochemical screening indicated that tannins, flavonoids, fatty acids, phenol, steroids, saponins and alkaloids were present. The seed extracts were tested against eight pathogenic organisms comprising of two Gram positive and two Gram negative bacteria; two fungi and two viruses using Agar and Disc diffusion methods. The plant extracts exhibited antimicrobial activities against all the tested organisms. This investigation therefore, suggests the incorporation of *Afzelia africana* and *Detarium microcarpum* seeds into human diets as they are rich in medicinal agents that could trigger great physiological effects. It also authenticates their use as soup thickeners in eastern Nigeria and in the production of snacks.

Capsule Summary: The phytochemical constituents of *Afzelia Africana* and *Detarium microcarpum* seed endosperms were investigated and tannins, flavonoids, fatty acids, phenols, steroids, saponins and alkaloids were present. They also exhibited antimcorbial activities against tested pathogenic organisms.

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INTRODUCTION

The search for new drugs has increased greatly due to increase in infectious diseases as well as drug resistance by pathogenic organisms. Man has continued to seek for inspiration from plants due to their diverse medicinal properties. Plants contain numerous chemical compounds of which many are biologically active and can be used in the prevention and treatment of diseases and infections. These biologically active compounds include flavonoids, tannins, saponins, alkaloids, glycosides and phenolics (Cowan, 1999).
Afzelia africana and *Detarium microcarpum* are leguminous plants of the caesalpinioceae family that are widely distributed in Africa and Asia (Keay et al., 1964; Hopkins and Stanfield, 1966). The seeds flour are used as soup thickener in eastern Nigeria (Igwe and Friday, 2017). Their fruits are edible and are rich in lipids, carbohydrates, proteins and vitamins (Nwokocha and Olorunsola, 2016; Oibiokpo et al., 2014). Their roots, leaves and barks have been utilized in traditional medicine for the treatment of various diseases and infections. *A. africana* is used in the treatment of constipation, diarrhea, hernia and malaria (Alyaa et al., 2015; Asase et al., 2005 Igoli et al., 2005). *D. microcarpum* is used for the treatment of meningitis, itches, diarrhea, dysentery and tuberculosis (Iwu, 1993). The antibacterial, antifungal, antihyperglycemic, anti-inflammatory and analgesic properties of *A. africana* and *D. microcarpum* leaves, roots

and barks have been reported (Akinpelu et al., 2008; Aiyegoro et al., 2011; Alyaa et al., 2015; Akah et al., 2007; Ajayi et al., 2017; Abubakar, 2017; David et al., 2017, Okolo et al., 2012; Rouamba et al., 2017).

The present study is aimed at investigating the phytochemical constituents and antimicrobial activities of *Afzelia africana* and *Detarium microcarpum* seed endosperms.

MATERIAL AND METHODS

Plant collection, preparation and extraction

Afzelia africana and *Detarium microcarpum* seeds were purchased from New market Aba, Abia State, Nigeria and authenticated at the Plant Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. *A. africana* seeds were roasted in hot sand and the coat removed using a small hammer. The seeds endosperm was milled using an electric blender and oven dried at 60°C for 3 hours. On the other hand, the seeds of *D. microcarpum* were soaked in clean water for 24 h to remove the ectocarp. The seed endosperms were then sun-dried for 48 h. They were milled using a blender.

Soxhlex extraction method was used. 20 g of the samples each were wrapped in a porous paper (What man No.1 filter paper). The wrapped sample was put in a soxhlex reflux flask containing 200 ml of petroleum ether. The upper end of the reflux flask was connected to a condenser. By heating the solvent in the flask through electro-thermal heater, it vaporizes and condensed into the reflux flask. The wrapped sample was completely immersed in the solvent and remained in contact with it until the flask filled up and siphoned over thus carrying the extracts from the sample down to the boiling flask. This process was allowed on repeatedly, for about 4 hours. The solvent was recovered using rotary evaporator and the extracts were dried in the oven at 60^0C for 3 minutes to remove any residual solvent.

Phytochemical screening

Methanol extract of the samples were used for the test. The dried sample were soaked in the solvent overnight and filtered before heating to one quarter volume of flask.

Alkaloid

The extract (1.0 ml) was shaken with 5.0 ml of 2% HCl on a steam bath and filtered. To 1 ml of the filtrate, Wagner's reagent (iodine in potassium iodide solution) was added. A reddish brown precipitate confirms that its presence.

Saponins

One millilitre of the filtrate was diluted in 1 ml of water and shaken vigorously. A strong frothing confirms presence of saponin.

Tannins

Five millilitres of the extract was added to 2.0 ml of 1% HCl. Deposition of a red precipitate shows the presence of tannin.

Steroids

The extract (1 ml) was dissolved in 2.0 ml of chloroform in a test-tube, and then 1 ml of conc. H_2SO_4 was added. Formation of reddish brown colour at the inter-phase confirms the presence of steroid.

Phenolics

The extract (1.0 ml) was added with 1.0 ml of 10% ferric chloride. The formation of a greenish brown or black precipitate or colour is taken as positive for a phenolic nucleus.

Flavonoids

The extract (1.0 ml) was diluted in 1.0 ml of diluted NaOH. Formation of precipitate shows the presence of flavonoid.

Fatty acids

Few drops of Sudan III reagent solution was added to 2 ml of the extract. Formation of reddish colour shows the presence of fatty acids.

Antimicrobial analysis

The test organisms for this study were bacteria namely *Eschrichia coli, Staphylococcus aureus spp, Salmonella spp* and *Streptoccocus pneumonia spp.; fungi include Candida albicans, Sporothix schenckii. Then virus include Hapetitis B and C viruses.* The pure clinical isolates were obtained from the Department of pathology laboratory, National Root Crops Research Institute (NRCRI) Umudike, Nigeria. All the clinical isolates were checked for purity and are maintained on Nutrient broth at 4^0C in the refrigerator until required for analysis.

Antibiotics

The antibiotics used as control were vancomycin for bacteria, fluconazole for fungi, lamuvidine for Hapetitis B and essential fort for Hepatitis C.

Standardization of bacteria cell suspension

The nutrient broth cultures of the organisms for this study were taken and inoculated on a fresh agar plate of nutrient agar for 24 hours. Sterile distilled water (2 ml) was poured on it and then mixed with the inoculums, 1ml of each was taken and were transferred into 9 ml of sterile distilled water and was diluted to 10^4 fold. One hundred micro litre of this was taken and poured on the surface of the agar and then spread evenly with the use of a spreader on the plate to be used for study. The different extracts of the sample were reconstituted with sterile distilled water and ethanol. The initial concentration of the plant extract (5 g) was diluted using 50 ml of sterile water and Ethanol to obtain the stock culture. From this stock culture, different concentration were gotten such as 100,150 and 200 mg/ml for each extracts.

Antimicrobial susceptibility assay

Table 1: Phytochemical screening result of *A. africana* and *D. microcarpum* seeds

Phytochemicals	*A. africana*	*D. microcarpum*
Tannins	+	+
Flavonoids	+	+
Fatty acid	+	+
Phenol	+	+
Steroids	+	+
Saponins	+	+
Alkaloids	+	+

+ = present; - = absent

Two methods were employed for the antimicrobial testing which are the Agar diffusion method and Disc diffusion method.

Agar diffusion method

The antimicrobial screening of the ether extract was done as described by Lino and Deogracious (2006). Nutrient and potato dextrose agar were poured in sterile Petri dishes and was allowed to solidify. 1 ml of the test culture was dropped on the solidified agar and the organism was spread all over the surface of the agar using a spreader. Wells of approximately 5 mm in diameter were made on the surface of the agar medium using a sterile cork borer. The plates were turned upside down and the wells labelled with a marker. Each well was filled with 0.2 ml of the extract. The plates were incubated aerobically at 37^0C for 24 hours. Sensitivity of the organisms to the extract was recorded.

Disc diffusion method

The locally prepared sterile discs were soaked in the water extract for some hours and nutrient agar medium was poured in sterile Petri Dishes and it was allowed to solidify. 1 ml of the test organisms was placed on the solidified agar and it was spread all over the surface of the agar. The soaked disc was picked using sterile forceps and it was dropped on the surface of the agar. The plates were incubated at 37^0C for 24 hours. Sensitivity of the organisms was recorded.

Statistical analysis

Data's were expressed as means ± SD (standard deviation) of four replicates and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by Duncan multiple test using statistical analysis software. Values were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The phytochemical screening of *A. africana* and *D. microcarpum* seed extracts confirmed the presence of tannins, flavonoids, fatty acid, phenol, steroids, saponins and alkaloids (Table 1).

Table 2, 3 and 4 shows the zone of inhibitions of *A. africana* seeds, *D. microcarpum* seeds and control antibiotics against the selected microorganisms respectively. *A. africana* seeds extract showed no zone of inhibition against *Salmonella spp.*, *E. coli, S. schenckii, Hapetitis* B and C viruses

Table 2: Zone of inhibition of *A. africana* seeds against selected microorganisms at different concentrations (mg/ml)

Test microorganism	100 mg/ml	150 mg/ml	200 mg/ml
S. aureus	2.54±0.08	4.37±0.07	6.47±0.03
S. pneumoniae	3.43±0.02	5.78±0.02	7.27±0.03
Salmonella spp.	_	6.27±0.04	8.42±0.25
E. coli	_	3.56±0.05	5.86±0.04
C. albicans	2.32±0.03	3.77±0.03	5.67±0.04
S. schenckii	_	2.27±0.04	4.55±0.07
Hapetitis B	_	1.62±0.35	3.49±0.01
Hapetitis C	_	2.77±0.35	3.12±0.17

Data are mean of three replicate determinations; - = not detected. Values were considered significant at $p<0.05$

Table 3: Zone of inhibition of *D. microcarpum* seeds against selected microorganisms at different concentrations (mg/ml)

Test microorganism	100 mg/ml	150 mg/ml	200 mg/ml
S. aureus	3.33±0.09	7.38±0.02	8.82±0.11
S. pneumoniae	2.36±0.08	6.33±0.02	8.45±0.07
Salmonella spp.	_	5.54±0.08	7.30±0.42
E. coli	_	5.32±0.10	8.32±0.10
C. albicans	1.82±0.03	3.77±0.03	4.87±0.03
S. schenckii	1.42±0.03	3.22±0.02	5.81±0.17
Hapetitis B	_	2.27±0.04	4.45±0.21
Hapetitis C	_	1.91±0.01	4.77±0.10

Data are mean of three replicate determinations. - = not detected. Values were considered significant at $p<0.05$

Table 4: Zone of inhibition of control antibiotics against selected microorganisms at different concentrations (mg/ml)

Test microorganism	100 mg/ml	150 mg/ml	200 mg/ml
S. aureus	9.52±0.10	13.15±0.49	18.51±0.12
S. pneumonia	8.81±0.15	14.27±0.53	19.52±0.10
Salmonella spp.	7.82±0.02	12.51±0.12	17.32±0.17
E. coli	7.56±0.05	13.38±0.02	15.39±0.12
C. albicans	8.67±0.10	13.77±0.03	15.79±0.01
S. schenckii	7.74±0.08	14.89±0.04	18.50±0.28
Hapetitis B	6.34±0.08	9.47±0.31	12.55±0.21
Hapetitis C	7.57±0.10	10.77±0.09	14.41±0.12

Data are mean of three replicate determinations. Values were considered significant at $p<0.05$

at 100 mg/ml concentration (Table2). The same was observed for *D. microcarpum* at the same concentration with the exception of *S. schenckii* (Table 3).

The phytochemical screening of *A. africana* and *D. microcarpum* seeds extracts revealed the presence of tannins, flavonoids, fatty acids, steroids, saponins, phenols and alkaloids (Table 1). These phytochemicals are known to be biologically active and good antimicrobial agents.

Tannins are naturally occurring high molecular weight water soluble polyphenolic compounds. The antibacterial, anticancer, antioxidant and anti-inflammatory activities of tannins has been reported (Chung et al., 1998; Doss et al., 2009; Scalbert, 1992; Ujwala et al., 2012). Latha et al., (2015) studied the antioxidant and anticancer activities of tannins isolated from the root bark of *Clerodendrum infortunatum* Linn. and reported that the isolated tannins exhibited significant antioxidant and antiproliferation effects against HCT-15 cell lines, while Akiyama et al., (2001) reported the antibacterial action of tannins against *S. aureus*. A. africana and D. microcarpum seeds can be used to fight diseases and infections caused by *S. aureus* and can also be used in the prevention of cancer and diabetes.

Flavonoids were detected in the seed extracts of A. africana and D. microcarpum. Flavonoids has been reported to exhibit various biological functions and medicinal properties such as anti-inflammatory, antioxidant, antibacterial, antiviral and cardioprotective properties (Anila and Vijayalakshmi, 2003; Manach et al., 2005; Vinson et al., 1995; Xu et al., 2000). Flavonoids exhibit antibacterial action by causing cell lyses of pathogenic bacteria (Gera et al., 2016). Foods rich in flavonoids are known to reduce the risk of heart diseases and also lower blood pressure in hypertensive patients (Galleano et al., 2012), thus suggesting the use of *A. africana* and *D. microcarpum* seed endosperms in the prevention/treatment of heart diseases and hypertension.

Fatty acids are important nutritious substances which must be incorporated into mans' diet for effective functioning of the body. Fatty acids and their derivatives has been reported to possess antimicrobial activities (Abdelillah et al., 2013; Desbois and Smith, 2010; Desbois, 2012; Dilika et al., 2000; Nitbam et al., 2016; Zheng et al., 2005). In a research conducted by Desbois and Lawlor (2013), they reported that long chain polyunsaturated fatty acids possessed antibacterial activity against *Propionibacterium*

acne and *S. aureus*, while Abdelillah et al., (2013), reported that fatty acid methyl ester fraction isolated from Algerian Linus usitatissimum L. seeds exhibited antifungal activity against *Aspergillus flavus* and *ochraceus*. They also attributed the observed antifungal potency to the abundance of linoleic and linolenic acids in the seed. The presence of fatty acids in *A. africana* and *D. microcarpum* seeds not only help in fighting diseases but are also nutritious substances involved in metabolism in living organisms and thus, are essential for human health.

Steroids were also detected. Steroids are hormones that control the development and function of the sexual organs and are used for the treatment of various diseases such as allergies, arthritis and diseases resulting from hormone deficiencies or abnormal production (Bhawani et al., 2010; Igwe and Okwu, 2013). *A. africana* and *D. microcarpum* seeds could be of great help in managing fertility problems, arthritis and allergies.

Saponins which are known to possess anti-inflammatory, anti-tumor, hapatoprotective and haemolytic activities (Moghimipour and Handali, 2015; Yassin et al., 2013) were also detected in the seed extracts of *A. africana* and *D. microcarpum*. The presence of alkaloids which are known to possess analgesic, antimalarial and antibacterial properties (Kurhekar, 2007; Wadood et al., 2013) in *A. africana* and *D. microcarpum* seed endosperms suggests their use in fighting headache, pains, malaria as well as infections.

A. africana and *D. microcarpum* seeds extracts were investigated for antimicrobial activities against eight pathogenic organisms comprising of two gram positive and two gram negative bacteria, namely, *Staphylococcus aureus*, *Streptococcus pneumoniae, Salmonella spp.* and *Escherichia coli*; two fungi namely, *Candida albicans* and *Sporothrix schenckii*, and two viruses, namely, *Hapetitis* B and C viruses. A clear zone of inhibition was observed against the tested pathogenic organisms. The highest zone of inhibition was against Salmonella spp. at 200 mg/ml concentration and the least against hapetitis C virus for *A. africana* (Table 2), whereas the highest zone of inhibition was against *S. aureus* at 200 mg/ml concentration and the least against hapetitis B virus for *D. microcarpum* (Table 3). For the control antibiotics, the highest zone of inhibition was against *S. pneumoniae* at 200 mg/ml concentration and the least against hapetitis B virus (Table 4).

The observed antimicrobial activities were due to the presence of tannins, flavonoids, fatty acids, saponins, steroids and alkaloids in *A. africana* and *D. microcarpum* seeds. The ability of the extracts to inhibit the growth of HBV and HCV suggests the use and incorporation of *A. africana* and *D. microcarpum* seeds in the diet of Hapetitis patients. In general, incorporating *A. africana* and *D. microcarpum* seeds into human diets could help prevent and fight diseases and infections.

CONCLUSIONS

The qualitative phytochemical screening of the seed extracts of *A. africana* and *D. microcarpum* confirmed that they are rich in medicinal agents such as alkaloids, tannins, flavonoids, saponins, steroids and fatty acids. The extracts exhibited antibacterial, antifungal and antiviral activities against the tested organisms and therefore suggests the use of *A. africana* and *D. microcarpum* seeds in the treatment/prevention of infections caused by these organisms as well as incorporating them into human diets. This study also authenticates the use of the plant parts as a whole as antimicrobial agents.

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