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Full Length Research Paper

Chemical composition, antimicrobial activity, proximate analysis and mineral content of the seed of *Detarium senegalense* JF Gmelin

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***Detarium senegalense* JF Gmelin (Caesalpiniaceae), commonly known as tallow tree, is used traditionally for the treatment of bronchitis, pneumonia, internal complaints and skin diseases in Tropical Africa. The seed is used as a soup thickener in Eastern Nigeria. Analysis of the petroleum ether extract of the seeds with GC-MS produced ten constituents of which oleic and linoleic acids were the most prominent (30.8 and 44.1% respectively). The extract showed significant antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus kristinae*, *Streptococcus faecalis*, *Shigella flexneri*, *Klebsiella pneumonia, Serratia marcescens* and antifungal activity against *Aspergillus flavus, Aspergillus niger* and *Penicillium notatum*. Proximate analysis revealed that the seeds contain 24.43% carbohydrate, 7.23% protein, 31.16% fiber, 5.89% moisture and 1.93% ash. Mineral content analysis revealed the concentrations of potassium (99.26 mg/g), calcium (71.11mg/g), magnesium (77.83 mg/g), sodium (55.26 mg/g), iron (30.21 mg/g), manganese (7.89 mg/g), zinc (5.26 mg/g) and copper (4.29 mg/g). These results show the nutritional value of the seeds of *D. senegalense* and justified its use in the traditional treatment of skin diseases.**

Key words: *Detarium,* GC-MS, antimicrobial, proximate analysis, mineral content.

INTRODUCTION

Detarium senegalense JF Gmelin (Caesalpinaceae) is a tree of up to 36 m high with highly buttressed bole and it is native to Tropical Africa. It is commonly known as tallow tree. The leaves are eaten as vegetable and are used traditionally as wash for itch, enema for dysentery and eye wash for conjunctivitis. The bark is used in the treatment of anaemia and to eject retained placenta. It is macerated in palm wine in Senegal for bronchitis, pneumonia and all internal complaints. The fruits which are globular and slightly flattened occur in two forms, one commonly known as ofo is popularly eaten and used as a soup thickener in Eastern Nigeria. They are given in the case of arrow poisoning. It is also used locally for the treatment of skin diseases (Burkill, 1995). A water soluble non-starch polysaccharide which suggests that it has nutritional properties has been reported in seed flour of the plant (Wang et al., 1996).

An anthocyanidin alkaloid (2-methoxyamine 3, 4, 5, 7-tetrahydroxy anthocynadine) with antibacterial activity has been isolated from the stem bark of the plant (Okwu and Uchegbu, 2009). A detarium meal was observed to elicit significant reduction in the plasma glucose levels of the human subjects investigated (Onyechi et al., 1998). The gum from the plant is reported to have shown promising antidiabetic effect in experimental rats (Adikwu

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et al., 2004). This paper reports for the first time, the chemical composition by GC/MS, antimicrobial activity, proximate analysis and the mineral composition of the seed of *D. senegalense* JF Gmelin. This investigation is useful to identify the bioactive compounds of the seed which may be responsible for its therapeutic properties and hence authenticate its traditional use.

MATERIALS AND METHODS

Plant material

The dried seeds of *D. senegalense* were purchased from herb sellers in Oyingbo Market in Lagos State, Nigeria. They were identified by Mr. Daramola at the Herbarium of the Department of Botany and Microbiology, University of Lagos and a voucher specimen (FHI 56829) was prepared and deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Akoka, Lagos, Nigeria.

Extraction procedure

D. senegalense seeds were oven-dried at 50°C overnight and ground to powder. The ground plant material (1 kg) was extracted cold with 3 L petroleum ether (60 to 80°C) for 48 h. The extract was filtered and concentrated *in vacuo* at 30°C using the rotary evaporator and stored in amber colored bottles at 8°C.

GC-MS analysis

GC-MS analyses were performed on a Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP-6890 gas chromatogram. The temperature and column conditions used are as follows: initial temperature of 70°C, maximum temperature of 325°C, equilibration time of 3 min, ramp of 4°C/min, final temperature of 240°C; inlet: split less, initial temperature of 220°C, pressure of 8.21 psi, purge flow of 30 ml/min, purge time of 0.20 min, gas type was helium; column: capillary was 30 m x 0.25 mm i.d., film thickness of 0.25 µm, initial flow of 0.7 ml/min, average velocity of 32 cm/s; MS: EI method at 70 Ev.

Identification of components

The components of the oil were identified by matching their mass spectra and retention indices with those of the Wiley 275 library (Wiley, New York) in the computer library and in literature (Shibamoto, 1987). The percentage composition of each component was calculated from summation of the peak areas of the total oil composition.

Antibacterial assay

The bacterial cultures used in this study were obtained from the Department of Biochemistry and Microbiology, Rhodes University, South Africa. They consisted of Gram-positive and Gram-negative strains. The minimum inhibitory concentration (MIC) values of the oil on each organism were determined using microplate dilution method (Ellof, 1998). Bacterial strains were cultured overnight at 37°C on Muller Hilton broth (MHB, BBL) and were adjusted to a final density of 10^6 cfu ml^{-1}. This was used to inoculate 96-well microtitre plates containing serial 5-fold dilutions of the oil (0.1 to 0.00625 v/v) under aseptic conditions. The oil was dissolved in 10% dimethylsulfoxide (DMSO). The plates were incubated under aerobic condition at 37°C and examined after 24 h. As an indication of bacterial growth, 40 µl of 0.2 mg ml^{-1} p-iodonitrotetrazolium (97% purity, Fluka Chemie) solution was added to each well and incubated for 30 min at 37°C. The colourless tretrazolium salt was reduced to a red-coloured product by the biological activity of the organisms. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of oil as indicated by a clear solution was required for it to be declared active (Ellof, 1998). Streptomycin and chloramphenicol were used as positive controls in the experiment with sample free solutions and 10% DMSO as the controls.

Antifungal assay

A total of three fungal species were used for the investigation. Each culture was maintained on potato dextrose agar (PDA) and was recovered for testing by subculturing on fresh PDA for three days. PDA plates were prepared by autoclaving before the addition of the filtered extracts. Each extract was then mixed with the molten agar to final concentrations of 0.00625, 0.0125, 0.025, 0.05 and 0.1 (v/v), were poured into Petri dishes and left overnight. Plates containing only PDA or PDA with 10% DMSO served as controls. The prepared plates containing the extracts were inoculated with plugs obtained from the actively growing margin of the fungal plates and incubated at 25°C for three days. Diameter of fungal growth was measured and expressed as means of percentage growth inhibition of three replicates (Afolayan and Meyer, 1997; Quiroga et al., 2001). Significant differences within the means of the treatments and the controls were calculated using the LSD statistical test (Steel and Torrie, 1960).

Proximate analysis

The Association of official analytical chemists (AOAC, 1990) recommended methods were adopted to determine the moisture, ash, crude fibre, total protein and carbohydrate. All analyses were carried out in triplicates. Moisture content was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 105°C; ash was determined by the incineration of 10.0 g samples placed in a muffle furnace maintained at 550°C for 5 h; protein (% total nitrogen x 6.25) was determined by the Kjeldahl method using 2.0 g samples; crude fibre was obtained by digesting 2.0 g of sample with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550°C for 5 h. Total carbohydrate was obtained by difference.

Mineral content

The powdered seeds (200 mg) were digested using 20 ml of concentrated HNO_3. The solution was then filtered into a 100 ml volumetric flask and made up to the mark with distilled water. Calcium, sodium, potassium, manganese, zinc, iron, copper and magnesium were determined by atomic absorption spectrophotometry (Analyst 200, Perkin Elmer, Waltham, MA, U.S.A).

RESULTS AND DISCUSSION

Ten compounds representing more than 99% of the fixed oil were identified in the chemical analysis carried out

Table 1. Chemical composition of the oil of *Detarium senegalense* seeds.

Number	Compound	Kovat index	Composition (%)
1	Cyclohexanone	630	1.4
2	β- Myrcene	1075	1.4
3	Cis-Rose oxide	1225	0.5
4	Camphor	1271	0.2
5	Citronellol	1383	8.7
6	E-Citral	1439	1.3
7	Isoledene	1565	1.4
8	Palmitic acid	2074	4.2
9	Linoleic acid	2271	44.1
10	Oleic acid	2275	30.8
	Yield (% v/w)	2.6	

Table 2. Antibacterial activity of the seeds of *Detarium senegalense*.

Bacteria	Minimum inhibitory concentration			
	Gram +/-	Oil (v/v)	Chloramphenicol[a] (µg/ml)	Streptomycin[b] (µg/ml)
Staphylococcus aureus	+	0.1	1.25	10.0
Staphylococcus epidermidis	+	0.1	0.3125	0.3125
Bacillus cereus	+	NA	12.5	10
Micrococcus kristinae	+	0.1	5	0.125
Streptococcus faecalis	+	0.1	1.25	5
Escherichia coli	_	NA	10	10
Pseudomonas aeruginosa	_	NA	10	12.5
Shigella flexneri	_	0.1	12.5	10
Klebsiella pneumoniae	_	0.1	1.25	5
Serratia marcescens	_	0.1	5	5

Minimum inhibitory concentration (ml/ml); NA, not active.
[a]Chloramphenicol in µg/ml, [b]Streptomycin in µg/ml.

(Table 1). Three fatty acids (palmitic, linoleic and oleic acids) were identified. Linoleic (44.1%) and oleic acids (30.8%) were the major components of the oil and these unsaturated fatty acids which have been reported to have antimicrobial activity may be responsible for the observed activity in the seeds (McGaw et al., 2002; Seidel and Tailor, 2004; Agoramoorthy et al., 2007). The anti-bacterial assay showed that the oil from the seed inhibited most of the Gram-positive bacteria with the exception of *Bacillus cereus* and three Gram-negative bacteria except *Escherichia coli* and *Pseudomonas aeruginosa* respectively (Table 2). The ethanolic extract of the stem bark of this plant has however been reported to inhibit the growth of *E. coli* and *P. aeruginosa* (Okwu and Uchegbu, 2009). The oil inhibited the growth of the Gram-positive and the Gram-negative bacteria at 0.1 (v/v).

The oil showed a range of antifungal activity against the three fungi tested (Table 3). At the highest concentration (0.1 v/v), the highest growth inhibition was observed on *Aspergillus niger* (70.46%) while *Aspergillus flavus* showed the least (55.33%). *Penicillium notatum* showed 68.43% growth inhibition at 0.1 v/v. The ability of the oil to inhibit the growth of most of the Gram-positive bacteria and some of the Gram negative bacteria as well as the fungal species is an indication of the possible broad spectrum antimicrobial abilities of the seed. Fixed oils from plants have been reported to have broad spectrum antimicrobial activity against some of the bacteria and fungi used in this work (Arici et al., 2005; Singh et al., 2007; Kaithwas et al., 2011). The result of the proximate analysis presented in Table 4 showed that *D. senegalense* seeds had low proportions of carbohydrate (28.43%), fiber (31.16%) and protein (7.23%). The moisture and ash content of the seeds fell within the range reported for seeds of other fruits (FAO, 1968). The low moisture content is an index of stability, quality and shelf life (Marangoni and Alli, 1988). The results obtained in this work are comparable with the proximate analysis results reported for the dehulled seeds of *Detarium microcarpum* except for the protein and crude fiber contents which were 37.1 and 2.9% respectively (Akpata and Miachi, 2001).

Table 3. Antifungal activity of the seeds of *Detarium senegalense.*

Concentration (v/v)	Growth Inhibition (%)		
	Aspergillus flavus	***Aspergillus niger***	***Penicillium notatum***
0.1	55.33^{e}	70.46^{f}	68.43^{f}
0.05	53.33^{e}	58.61^{e}	59.72^{e}
0.025	50.00^{d}	54.72^{d}	55.00^{d}
0.0125	43.89^{c}	40.48^{c}	50.00^{c}
0.00625	30.00^{b}	35.28^{b}	41.94^{b}
Control	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.025	0.02	0.013

Values are means of percentage growth inhibition of three replicates: values within a column followed by the same superscript of the same species are not significantly different at $p < 0.05$ according to the LSD test. LC_{50} values are in ml/ml.

Table 4. Proximate composition of seeds of *Detarium senegalense.*

Sample	Value
Moisture (%)	5.89 ± 0.12
Ash (%)	1.93 ± 0.08
Carbohydrate (%)	28.43 ± 0.10
Protein (%)	7.23 ± 0.07
Fiber (%)	31.16 ± 1.50

All data are mean ± standard deviation of triplicate determinations.

Table 5. Mineral composition of seeds of *Detarium senegalense* (mg/g of dry matter).

Element	Concentration
Potassium	99.26
Magnesium	77.83
Calcium	71.11
Sodium	55.26
Iron	30.21
Manganese	7.89
Zinc	5.26
Copper	4.99

Values are means of duplicate determinations.

Table 5 shows the micro and macro-elements present in the studied seeds. All the four macro-elements investigated were found in the seed with potassium occurring in the largest amount and sodium in the least. These macro-elements play vital roles in the metabolism of living organisms. Potassium and sodium maintain the water balance in cells and are important for the transmissions of the nerve impulse, as well as the stimulation of the normal movement of the intestinal tract (Smith, 1986). Magnesium maintains, repairs cells, provides energy and increases the body's resistance to infection. It also plays a role in the metabolism of calcium (Glew et al., 1997). Its deficiency may result in nervousness, convulsions, anaemia, insomnia and vertigo (Al-Ghamdi et al., 1994). Calcium provides rigidity to the skeleton and the calcium ion plays a role in many metabolic processes (FAO/WHO, 1998). In the micro-elements investigated, iron occurred in the largest amount and copper in the least. Iron helps in oxygen transport and oxidative metabolism in the body and assist in blood formation. Iron deficiency can lead to the impairment of immunologic responses as well as phagocytic action of neutrophyl leucocytes (Scrimshaw, 1984). The seed of *D. senegalense* is however rich in iron when compared with other food seeds and the iron content in some standardized Nigerian dishes (Onabanjo and Oguntona, 2003). Zinc helps in the synthesis of tryptophan which is an essential amino acid while copper is the main component of respiratory pigments and its deficiency leads to anaemia and graying of hair.

Conclusion

The results obtained in this study show that the oil from the seed of *D. senegalense* has antimicrobial activity and contains some active principles which may be responsible for the observed activities. The important minerals found in the seeds may also be major contributors to the medicinal and nutritional value of the plant. These results support the use of the seeds traditionally, for the treatment of skin diseases.

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