



Phytochemical analysis and antimicrobial activity of fruit of *Detarium microcarpum*

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Abstract

The genus *Detarium* is indigenous to Africa. In west Africa the genus is represented by eight species, however only three species *D. macrocarpum*, *D. microcarpum* and *D. senegalense* are of ethnomedicinal and pharmacological interest. *Detarium* species are widely and commonly used in traditional medicine in the treatment of diverse ailments, including, fever, malaria, bronchitis, convulsions, diabetes, microbial infections, etc. the present study was carried out to detect the presence of some phytochemical constituents in the fruit pulp, and to test the antimicrobial activities of n-hexane, chloroform, ethyl acetate and ethanol fruit pulp extract. The GC/MS analysis revealed the presence of the n-hexadecanoic acid, cedarn-diol, (8S, 14)-, (-)-globulol, benzoic acid, (E)- 9-octadecenoic acid, oleic acid and hexadecanoic acid, methyl ester. The antimicrobial test of the fruit pulp extract showed activity against *Pseudomonas aeruginosa*, but no activity against *Staphylococcus aureus* and *Aspergillus niger*.

Keywords: Phytochemical analysis, antimicrobial activity, *detarium microcarpum*

Introduction

Plants are known to provide a rich source of raw materials for traditional medicine in Africa and other developing countries because they are readily available to the local populace. Traditional medicine is sum total of knowledge and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, to treat physical and mental illness (Mahmoodally, 2013) [13]. In many cultures there is this belief that every plant growing on the surface of the earth has a medicinal property or use. Drugs have been derived from plant extracts which are in turn utilized by herbalists who are familiar with those species with marked biological activity and are known to have bioactive molecules against infections caused by fungi, bacteria, viruses and protozoa. Traditional medicine could be regarded as total combination of knowledge and practices whether applicable or not, used in the diagnosis, prevention or elimination of physical, mental or other diseases and which may rely exclusively on observations and past experiences handed down from one generation to another verbally or in writing. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (sharma *et al.*, 2012) [21].

The World Health Organization (WHO) reported that 80% of the emerging world's population relies on traditional medicine for therapy. During the past decades, the developed world has also witnessed an ascending trend in the utilization of Complementary of Alternative Medicine, particularly herbal remedies (Chintamunne and Mahmoodally, 2012). Herbal medicines include herbs,

herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients. While 90% of the population in Ethiopia use herbal remedies for their primary healthcare, surveys carried out in developed countries like Germany and Canada tend to show that at least 70% of their population have tried Complementary alternative medicine at least once (Gurib-Fakim, 2006, Chintamunne and Mahmoodally, 2012) [9]. It is likely that the profound knowledge of herbal remedies in traditional cultures, developed through trial and error over many centuries, along with the most important cures was carefully passed on verbally from one generation to another. Indeed, modern allopathic medicine has its roots in this ancient medicine, and it is likely that many important new remedies will be developed and commercialized in the future from the African biodiversity, as it has been till now, by following the leads provided by traditional knowledge and experiences (Gurib-Fakim, 2006, Chintamunne and Mahmoodally, 2012, Nunkoo and Mahmoodally, 2012 Shohawon and Mahmoodally, 2013) [7, 9, 13, 14, 22]. The genus *Detarium* (Fabiaceae, Sub family Caesalpinaceae) is represented by 8 species, however only 3 species *D. macrocarpum*, *D. microcarpum*, *D. senegalense* are of ethno medicinal and pharmacological interest (Akah *et al.*, 2012) [3]. These Species are very similar morphologically but appear to differ in ecological distribution. *Detarium* species are widely and commonly used in traditional medicine in the treatment of diverse ailments, including, fever, malaria, bronchitis, convulsions, diabetes, microbial infections, etc. Some pharmacological studies have been carried out to authenticate some of these claims. Phytoconstituents with

biological activities have been isolated from the genus (Akah *et al.*, 2012) [3]. The present study aimed at the studying composition of the chloroform extract of the fruit pulp of *D. microcarpum* and testing the antimicrobial activity of the fractions obtained from the ethanol extract of the fruit pulp.

Materials and Methods

Materials

Study design

This study is a laboratory-based descriptive study.

Study Area

The fruits of *D. microcarpum* were obtained from Alobayid market in Kordofan state. The extraction was carried out in the phytochemistry laboratory at Nile College. Microbiological tests were conducted at the National Health Laboratory (Khartoum).

The GCMS analysis of the chloroform extract was carried out at University of Medical Sciences and Technology (Khartoum).

Plant material

The plant material (*D. microcarpum* fruits) was brought from Kordofan.

Solvents

Ethanol, Chloroform, Hexane, Petroleum ether, Ethyl acetate, Sodium bicarbonate, Potassium hydroxide, Diethyl ether

Equipment and instrument

Soxhlet, UV lamp, TLC plates, oven, water bath, pre-coated silica gel plates, electronic balance, separating funnel, mortar and pestle and laboratory glassware.

Reagents and Culture Media

Peptone water for preparation of microbial suspension
Mueller Hinton Agar for culturing bacteria for carrying the *in vitro* sensitivity testing
Sabouraud Dextrose Agar for culturing yeasts the *in vitro* sensitivity testing.

Methods

Extraction of the fruit pulp

About 64g of dry fruit pulp were extracted with 250ml of chloroform (3×250). The combined extracts were filtered through filter paper. The solvent was removed on Soxhlet and water bath. About 100ml of 5% NaHCO₃ were added to the residue in separatory funnel and shaken with 250ml of chloroform. The NaHCO₃ layer was separated and acidified with HCl and extracted with CHCl₃ then was dried over anhydrous Na₂SO₄ and sent for GC/MS analysis.

For antimicrobial test

30g of the plant material (fruit pulp) were extracted with 70% ethanol and filtered while hot. the ethanol extract was concentrated on water bath to remove the ethanol. The residue was fractionated on separatory funnel into hexane, chloroform and ethyl acetate fractions. the solvents were removed from the fractions and sent for antimicrobial test.

GC/MS method of analysis

GC/MS analysis of the sample was carried out by using GM/MS technique model (GC/MSQP2010-Ultra)

'Shimadzu Company, and capillary column (Rtx-5ms, 30m×0.25mm). The sample was injected by using split mode, helium as the carrier gas flow rate 1.61 ml/min. as final temperature Cowith rate 10c/min to 300 Coure program was started from 60he temperatTion source, theCoection port temperature was 300degree with 3 minutes hold time, the inj.The sample was analyzed by Cotemperature was 250 and the interface Cotemperature was 200using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library of the National Institute of Standards and Technology (NIST), results were recorded.

Antimicrobial Methods

Antibacterial activity evaluation

Antibacterial activity of endophytic fungi crude ethyl acetate extracts was evaluated by the agar disc diffusion method (Mothana and Lindequist, 2005). After the plates were solidified the freshly prepared microbial broth culture suspension (about 0.1 ml) was spread over the Mueller Hinton Agar media using L-shaped sterilized glass spreader under aseptic condition using laminar air flow. Blank paper discs (Schleicher & Schuell, Spain) with a diameter of 5.0mm were impregnated with 20 µl of 10 mg/ml crude extracts. After 5 min, antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 h at 37° C in the incubator. After incubation, the diameters of clear zone of inhibition produced around the discs were measured in mm and the plates were photographed. The solvent dimethyl sulfoxide (DMSO) was used as a negative control, while amoxicillin was used as a positive control. Three replicates were carried out for each extract against each of the test organisms.

Antifungal activity evaluation

Antifungal activity was also evaluated by the disc diffusion method (Mothana and Lindequist, 2005). The inoculums was evenly spread on the surface of 10 cm Petri dishes containing Sabouraud Dextrose Agar medium and exposed to dry. Then, the paper discs were impregnated with 20 µl of 10 mg/ml crude extracts. After 5 min, antifungal discs were dispensed onto the surface of the inoculated agar plates, after which the plates were incubated at 25° C for 24 h. After the colonies grew, the zones of inhibition around the disks were measured and recorded. DMSO was used as a negative control.

Ethical considerations

The ethical approval for conducting this study was obtained from The Ethical Committee Board of Nile College. Approvals to collect control strains and clinical isolates as well as to conduct microbiological investigations was obtained from the administrative bodies (Ministry of Agriculture, Khartoum State Laboratory).

Strengths and limitations

Few local studies have been done on the fruit of *Detarium microcarpum*, therefore this study will provide significant Information on the chemical constituents and antimicrobial activity of this plant that grow abundantly in the specified area.

Disseminations of results: The results will be disseminated through presentation and possibly publications replicates were carried out for each extract against each of the test organisms.

Results and discussion

The GCMS analysis of the chloroform extract showed the presence of fifty one components as shown in table 1.

Table 1: GC/MS results of the chloroform extract

No.	Name	Rt	Area%
1.	1-Butoxy-2-propanol acetate	3.465	0.12
2.	2-Propanethiol	3.530	0.49
3.	Phosphine, bis(1-methylethyl)-	3.704	0.12
4.	5,6-Dimethyl-6-hydroxytetrahydro-1,3-thiazin-2-thione	3.755	0.05
5.	Propane, 1,2-dichloro-2-methyl-	3.967	0.15
6.	2-Butanol, 1,4-dichloro-	4.152	0.30
7.	2,2-Bis(chloromethyl)-1-propanol	4.884	0.04
8.	Propane, 1,1-dichloro-	4.918	0.17
9.	Ethane, hexachloro-	5.521	0.15
10.	Lactic acid	6.821	0.04
11.	Propanal, 2,3-dichloro-2-methyl-	6.873	0.07
12.	Benzoic acid	7.081	2.11
13.	2,3-Dichloro-7-methylenebicyclo[2.2.1]heptanes	7.360	0.35
14.	n-Decanoic acid	9.765	0.11
15.	Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-	10.121	0.03
16.	Bicyclo[4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl-	10.490	0.13
17.	.alpha.-Muurolole	11.594	0.31
18.	Lanceol, cis	11.837	0.20
19.	trans-calamenene	11.918	0.54
20.	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	12.035	0.11
21.	1H-Indene, 3-(bromomethyl)-1,1-dimethyl-	12.196	0.20
22.	Aromadendrene oxide-(2)	12.553	0.39
23.	.beta.-Guaiene	13.386	0.18
24.	Andrographolide	13.497	0.25
25.	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	13.859	0.28
26.	Tetradecanoic acid	14.667	0.70
27.	2-Pentadecanone, 6,10,14-trimethyl-	15.546	0.22
28.	Hexadecanoic acid, methyl ester	16.392	1.40
29.	cis-9-Hexadecenoic acid	16.661	0.69
30.	n-Hexadecanoic acid	16.884	20.93
31.	Hexadecanoic acid, ethyl ester	17.091	3.27
32.	Isopropyl palmitate	17.386	0.98
33.	9-Octadecenoic acid, (E)-	18.618	1.59
34.	Oleic Acid	18.658	1.51
35.	Octadecanoic acid	18.813	2.50
36.	Lupeol	19.816	1.03
37.	1-Cyclohexene, 1,3,3-trimethyl-2-(1-methylbut-1-en-3-on-1-yl)-	19.885	0.65
38.	Naphthalene, decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methylene-4-pentenyl)-, [4aS-(4a.alpha.,5.alpha.,8a.beta.)]-	20.324	2.38
39.	(3-Methylphenyl) methanol, 3-methylbutyl ether	20.858	8.43
40.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	21.223	16.24
41.	5-(7a-Isopropenyl-4,5-dimethyl-octahydroindene-4-yl)-3-methyl-pent-2-en-1-ol	21.407	12.19
42.	-1-yl)-1-propen-1-methyl-(2-2-methyl-1-methylen-4Cycloheptane, vinyl-	21.675	2.65
43.	Androst-4-ene-3,6,17-trione	22.007	1.19
44.	Cedran-diol, (8S,14)-	22.232	5.75
45.	(-)-Globulol	22.718	2.62
46.	Tetrapentacontane, 1,54-dibromo-	23.262	1.14
47.	Octadecane, 1-chloro-	24.788	1.04
48.	1-Heptacosanol	24.865	0.58
49.	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	25.686	2.40
50.	Dotriacontane	26.611	0.48
51.	1-Hexacosanol	26.750	0.55

Seven components of the chloroform extract were identified. These are n-hexadecanoic acid, cedran-diol, (-)-globulol (overlapping with unknown), alpha-selinene, isopropyl palmitate, benzoic acid, (E)-9-octadecenoic acid, oleic acid and hexadecanoic acid, methyl ester.

The major components were found to be fatty acids and their esters and sesquiterpenes.

The mass spectra of some of the identified components are given in the appendices.

Antimicrobial activity results

The results of antimicrobial activity of the n-hexane, chloroform, ethyl acetate and ethanol fractions is given in table 2.

Table 2: Antimicrobial test result (inhibition zones for each microorganism)

Microorganisms (Clinical isolate)	10mg/ml A	10mg/ml B	10mg/ml C	10mg/ml D	5mg/ml A	5mg/ml B	5mg/ml C	5mg/ml D
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	0	23	0	0	0	15	0	0
<i>Aspergillus niger</i>	0	0	0	0	0	0	0	0

A: n-Hexane, B: Chloroform, C: Ethyl Acetate, D: Ethanol

Only the chloroform fraction of the ethanol extract of the pulp showed high activity against *Pseudomonas aeruginosa* (inhibitory zone 23mm) at concentration 10mg/ml. at 5mg/ml the same fraction showed moderate activity (inhibitory zone 15mm) against the same organism.

The GC/MS analysis of the chloroform extract of *Detarium microcarpum* fruit pulp showed the presence of fatty acids and their ester derivatives and sesquiterpenes as major components.

The presence of sesquiterpenes in the fruit pulp *D. microcarpum* has been reported in literature (Cavin *et al.*, 2006) [6], However the presence of clerodanes which have been reported to be present in the fruit pulp was not shown in the chloroform extract by GC/MS analysis.

To our knowledge, the antimicrobial test of the fruit pulp has not been reported. The high activity of the chloroform fraction of the aqueous ethanolic extract (23mm inhibition zone) against *P. aeruginosa* calls for further studies with a view to make use of the fruit pulp in developing a pharmaceutical preparation to be used against the microorganism

Conclusion

The GC/MS analysis of the chloroform extract of *Detarium microcarpum* fruit pulp showed the presence of fatty acids and their ester derivatives and sesquiterpenes as major components.

Only the chloroform fraction of the ethanol extract of the pulp showed high activity against *pseudomonas aeruginosa* (inhibitory zone 23mm) at concentration 10mg/ml. at 5mg/ml The same fraction showed moderate activity (inhibitory zone 15mm) against the same organism.

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Competing interests

The authors declare that they have no competing interests.

Author's contributions

The manuscript was carried out, written, and approved in collaboration with all authors.

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