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Chemical compositions of ethanolic extract and their fractions of *Acacia ehrenbergiana* aerial parts from Sudan

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Abstract

Acacia ehrenbergiana is a species of drought-resistant small tree found in the Sahara, northern Sahel, East Africa and Arabia. It is commonly known as "Salam". It is used in Sudanese Traditional Medicine for treatment of various ailments and diseases. The objectives of this work is to provide answers to impending research questions on compounds present in ethanolic extract and their partitioned solvents, dichloromethane and ethyl acetate fractions of the aerial parts of *Acacia ehrenbergiana* using GC/MS technique. Phytochemical analysis of plant extract and their fractions was performed by using GC/MS. Various constituents were identified after matching their mass fragmentation pattern with data available in GC/MS library of National Institute of Standards Technology (NIST). The ethanolic extract and dichloromethane fraction of aerial parts were dominated by 4-O-Methylmannose (43.34% and 45.20%) and 1,2,3-Benzenetriol (38.97% and 47.81%), respectively, as the main constituents, while the ethyl acetate fraction was dominated by phenol,2,2-methylene, bis{6-(1,1-dimethyl (23.15%), arachidic acid (21.29%) and hexane,2,3,4-trimethyl- (11.5%). In this study, the constituents of ethanolic extract and their fractions of *Acacia ehrenbergiana* aerial parts were chemically investigated using GC/MS technique. The resulted compounds provide evidence for the utilization of the plant as alternative natural resource of medicinal and industrial interest.

Keywords: *Acacia ehrenbergiana*; chemical composition, GC/MS, aerial parts

Introduction

Acacia ehrenbergiana is a species of drought-resistant bush or small tree found in the Sahara, northern Sahel, East Africa and Arabia. It is commonly known as "Salam". *A. ehrenbergiana* Gum exudate contain 4-O-Methylglucuronic acid; glucuronic acid, galactose, arabinose, and rhamnose [1]. Fatty Alcohols: Cosanol, tricosanol, tetracosanol, pentacosanol, hexacosanol, octacosanol, triacontanol and dotriacontanol Hydrocarbons: Hexadecane, octadecane, eicosane, docosane, tricosane, tetracosane, pentacosane and hexacosane. Sterols: Cholesterol, campesterol, stigmasterol and α -spinasterol Fatty Acids: Myristic, pentadecanoic, palmitic, margaric, stearic, oleic, and linoleic acids [2] and Saponins [3]. *Acacia* species are used in folk medicine as antidiarrhea, anti-amoebic, hypotensive, anti-diabetic and anti-inflammatory [4]. The plant showed anti-inflammatory and molluscicidal, hypotensive, anti-bacterial and wound healing activities [5]. This study aim to determine chemical constituents of ethanolic extract and their partitioned solvents, dichloromethane and ethyl acetate fractions of aerial parts of *Acacia ehrenbergiana*.

Materials and Methods**Plant Material**

The plant *Acacia ehrenbergiana* aerial parts was collected in April 2017 from Omdurman Islamic University Campus (Omdurman South, Khartoum State). The plant material was taxonomically identified by Prof. Maha Kordofani and Dr. Adam Mohammed Ahmed Bashir, Botany Department, Omdurman Islamic University.

Sample Preparation

Plant sample was carefully dried in the shade at room temperature. The dried aerial parts was ground into fine powder with a mechanical crusher and the powder was kept in glass container at room temperature.

Plant Extraction

Pulverized fine powder (50 g) was extracted with an 70% ethanol solution (v/v) by maceration extraction method for 5 days, the extracted solution was filtered with Whatmann No. 1 filtering paper twice to remove insoluble matrices. The extraction process was conducted three times to retrieve more of the active compounds. After the three extractions, the extracts were subjected to concentration by a rotary vacuum evaporator. Then, an 70% ethanol sample was set aside, and the remaining concentrates of 70% ethanol was dissolved by water and then partitioned with dichloromethane and ethyl acetate. Each sample was stored in a refrigerator until further analysis.

Gas-Chromatography-Mass Spectrometer (GC/MS)

The GC/MS analysis of the crude ethanolic extract of *A. ehrenbergiana* and their fractions was performed on a GC/MS - QP 2010, Shimadzu. GC/MS instrument equipped with reference libraries. Packed material for column were 50% phenyl and 50% methyl polysiloxane, column length 30 meter, diameter 0.025 mm, the flow rate of helium as carrying gas was 1 ml/min, the temperature of program consisted of 60-270 C, at rate of 4 C/min. MS were taken at ionization voltage 70 EV. Library search was carried out using wiley GC/MS library.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

GC/MS Analysis of *A. ehrenbergiana* aerial parts

(a) ethanolic crude extract: *Acacia ehrenbergiana* aerial parts ethanolic crude extract showed twelve constituents (Table 1). The major constituents were 4-O-Methylmannose (43.34%), 1,2,3-Benzenetriol (38.97%), phytol (2.41%), n-Hexadecanoic acid (1.90%) and Lanosterol (1.80%).

Five fatty acids compounds were identified in the ethanolic extract (3.52%). The results revealed that palmitic acid (1.190%) was formed as major component. Four sterols/terpenes compounds were identified (5.7%) and phytol (2.41%) was the major component.

(b) dichloromethane fraction: *Acacia ehrenbergiana* aerial parts Dichloromethane fraction showed thirteen constituents

(Table 2). The major constituents were phenolic compound, 1,2,3-Benzenetriol (47.81%), and polysaccharide, 4-O-Methylmannose (45.20%).

Five acids compounds were identified in the dichloromethane fraction of *A. ehrenbergiana* (3.23%). The results revealed that palmitic acid (1.90%) was formed as major component. Four sterols/terpenes compounds were identified (3.53%) and phytol (2.15%) was the major component.

(c) ethyl acetate fraction: *Acacia ehrenbergiana* aerial parts ethyl acetate fraction showed twelve constituents (Table 3) were Phenol, 2,2-methylenbis(6-(1,1-dimethyl (23.15%), Arachidic acid (21.29%), Hexane, 2,3,4-trimethyl- (11.5%), phytol (7.44%), Decane, 3,7-dimethyl (7.17%) as the major constituents.

Nine hydrocarbons compounds were identified in the ethyl acetate fraction (48.11%). The results revealed that hexane, 2,3,4-trimethyl (11.15%) followed by Decane, 3,7-dimethyl- (7.17%) were formed as major constituents.

One fatty acid compound was identified, Eicosanic acid (Arachidic acid) (21.29%) and one phenolic compound was identified, phenol, 2,2-methylenbis(6-(1,1-dimethyl (23.15%), while one terpene compound was identified (7.44%).

Uses of some bioactive compounds present in *A. ehrenbergiana* ethanolic extracts and their fractions:

Pyrogallol had highly cytotoxic effect on human lung cancer lines and less effect on human bronchial epithelium cell line [6]. Palmitic acid is used in cosmetics [7]. Phytol can be used as a precursor for the manufacture of synthetic forms of vitamin E and K1 [8]. Linoleic acid is used in beauty products industry because of its beneficial properties on the skin [9].

Alpha-linolenic acid is a type of omega 3. It reducing the risk of heart disease and heart attacks, reducing the risk of Hardening of the arteries (atherosclerosis) and high blood pressure [10].

Stearic acid produces dietary supplements and often used to coat metal powders such as aluminum and iron, this prevents oxidation, allowing compositions to be stored for a longer period of time [11]. n-cetyl alcohol is used in cosmetic industry as an emollient, emulsifier or thickening agent in the manufacture of skin creams and lotion [12].

Oleamide is used in cosmetics [13]. Stigmasterol is used as a precursor in the manufacture of semi-synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue re-building mechanisms related to estrogen effectors, as well as acting as intermediate in the biosynthesis of androgens, estrogen and corticoids. Also is used as the starting raw material for the synthesis of cortisol [14].

Table 1: Chemical compounds from *Acacia ehrenbergiana* aerial parts of ethanolic crude extract

Sr. No.	R. time	Name of compound	Other Names	Molecular Formula/chemical class	Molecular weight	Peak Area %
1	14.479	1,2,3-Benzenetriol	Pyrogallol	C ₆ H ₆ O ₃ Phenol	126.1100	38.97
2	19.713	4-O-Methylmannose	_____	C ₇ H ₁₄ O ₆ polysaccharide	194.183	43.34
3	22.325	Lanost-8-en-3-ol,(3.beta)-	(3b)-Lanost-8-en-3-ol	C ₃₀ H ₅₂ O Sterol/terpene	470.77	0.96
4	23.325	n-Hexadecanoic acid	Palmitic acid	C ₁₆ H ₃₂ O ₂ Fatty acid	256.4241	1.90
5	23.464	Hexadecanoic acid, ethyl ester	_____	Fatty acid	_____	0.46
6	24.465	n-Nanodecanol-1	_____	Terpene	_____	0.53
7	24.793	Phytol	(E)Phytol	C ₂₀ H ₄₀ O Terpin	296.5310	2.41
8	25.025	9,12-Octadecadienoic acid (Z,Z)	Linoleic acid	C ₁₈ H ₃₂ O ₂ Fatty acid	280.4455	0.52
9	25.025	9,12,15-Octadecatrienoic, (Z,Z,Z)	Linolenic acid	C ₁₈ H ₃₀ O ₂ Fatty acid	278.4296	0.43
10	25.276	Octadecanoic acid	Stearic acid	C ₁₈ H ₃₆ O ₂ Fatty acid	284.4772	0.21
11	27.129	Lanosterol	Lanosta-8,24-dien-3-ol	C ₃₀ H ₅₀ O Terpene	426.729	1.80
12	29.647	9,19-Cyclolanost-25-en-3-ol,methyl-,(3)	_____	Terpene	_____	0.10

Table 2: Chemical compounds from *Acacia ehrenbergiana* aerial parts dichloromethane fraction

Sr. No.	R. time	Name of compound	Other Names	Molecular Formula/chemical class	Molecular weight	Peak Area %
1	14.479	1,2,3-Benzenetriol	Pyrogallol	C ₆ H ₆ O ₃ Phenol	126.1100	47.81
2	19.713	4-O-Methylmannose	NSC403460	C ₇ H ₁₄ O ₆ Polysaccharide	194.183	45.20
3	22.136	1-Hexadecanol	n-cetyl alcohol	C ₁₆ H ₃₄ O Alcohol	242.4406	0.18
4	23.325	N-Hexadecanoic acid	Palmitic acid	C ₁₆ H ₃₂ O ₂ fatty acid	256.4241	1.90
5	23.464	Hexadecanoic acid, ethyl ester	_____	Fatty acid	_____	0.46
6	24.465	n-Nanodecanol-1	_____	Terpine	_____	0.53
7	24.793	Phytol	(E)Phytol	C ₂₀ H ₄₀ O Terpene	296.5310	2.15
8	25.025	9,12-Octadecadienoic acid (Z,Z)	Linoleic acid	C ₁₈ H ₃₂ O ₂ Fatty acid	280.4455	0.23
9	25.025	9,12,15-Octadecatrienoic, (Z,Z,Z)	Linolenic acid	C ₁₈ H ₃₀ O ₂ Fatty acid	278.4296	0.43
10	25.276	Octadecanoic acid	Stearic acid	C ₁₈ H ₃₆ O ₂ Fatty acid	284.4772	0.21
11	25.557	Octadecanoic acid, ethyl ester	Ethyl Octadecanoate	C ₂₀ H ₄₀ O Fatty acid	312.5304	0.17
12	27.258	9-Octadecenamide,(Z)-	Oleamide	C ₁₈ H ₃₅ NO Amide	281.4766	0.19
13	29.056	Stigmasterol	B-Stigmasterol	C ₂₉ H ₄₈ O Steroid	412.6908	0.68

Table 3: Chemical compounds from ethyl acetate fraction of *Acacia ehrenbergiana* aerial parts

Sr. No.	R. time	Name of compound	Other Names	Molecular Formula/chemical class	Molecular weight	Peak Area %
1	3.085	Hexane,2,3,4-trimethyl-	Pentane,2,3,-trimethyl-	C ₉ H ₂₀ Hydrocarbon	128.2551	11.15
2	3.547	Heptane,2,3-dimethyl-	_____	C ₉ H ₂₀ Hydrocarbon	128.2551	1.85
3	3.648	Octane,4-methyl-	_____	Hydrocarbon	_____	6.49
4	7.196	Decane,3,7-dimethyl-	_____	C ₁₂ H ₂₆ Hydrocarbon	170.3348	7.17
5	7.313	Dodecane	Adacane	C ₁₂ H ₂₆ Hydrocarbon	170.3348	3.07
6	8.255	Nonane,4,5-dimethyl-	5,6-Dimethyl-nonane	C ₁₁ H ₂₄ Hydrocarbon	165.3083	3.00
7	11.872	Dodecane,2,6,11-trimethyl-	_____	C ₁₅ H ₃₂ Hydrocarbon	212.4146	5.57
8	12.807	Hexadecane	n-Cetane	C ₁₆ H ₃₄ Hydrocarbon	226.4412	5.74
9	20.401	Eicosane	n-icosane	C ₂₀ H ₄₂ Hydrocarbon	282.5475	4.07
10	23.137	Eicosanoic acid	Arachidic acid	C ₂₀ H ₄₀ O ₂ Fatty acid	312.5304	21.29
11	24.793	Phytol	(2E)(7R,11R)-3,7,11,15-tetramethylhexadec-2-en-ol	C ₂₀ H ₄₀ Terpene	296.5310	7.44
12	27.843	Phenol,2,2-methylenbis{6-(1,1-dimethyl	_____	Phenol	_____	23.15

Conclusion

The *Acacia* species would serve as good source of pharmaceutical drugs. Quantitatively, the *Acacia ehrenbergiana* aerial parts ethanolic crude extract was characterized by abundance of 4-O-Methylmannose (43.34%), 1,2,3-Benzenetriol (38.97%), the Dichloromethane fraction was characterized by abundance of 1,2,3-Benzenetriol (47.81%) and 4-O-Methylmannose (45.20) while the ethyl acetate fraction was characterized by abundance of Phenol,2,2-methylenbis{6-(1,1-dimethyl (23.15%) and Arachidic acid (21.29%). Phytol was the most abundant terpene in ethanolic crude extracts of plant. The resulted compounds provide evidence for the utilization of the plant as alternative natural resource of medicinal and industrial interest.

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