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Original Article

A COMPARATIVE STUDY OF CHEMICAL COMPOSITION OF *ACACIA SEYAL* STEM, STEM WOOD AND STEM BARK DRY DISTILLATES USED BY SUDANESES WOMEN AS COSMETIC AND MEDICINE

IKRAM MOHAMED ELTAYEB[1*], ITMAD AWAD ELHASSAN[2], JIHAD HASAB ELRASOUL[1], EIMAN SALAH ELDIN[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan, [2]Pharmaceutical Industries Department, Industrial Research and Consultancy Centre, Ministry of Science and Technology, Khartoum, Sudan
Email: kramela_07@yahoo.com

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ABSTRACT

Objective: The content and chemical composition of dry distillates of the stem, stem wood and stem bark of *Acacia seyal* were investigated. The distillates are fumigants of *A. seyal* locally known in Sudan as *Dokhan* and widely used for its cosmetic, aromatic and medicinal value for the treatment of candidiasis, genital yeast infection, urinary tract infection, diarrhoea, respiratory tract infection, skin infection and with potent, antioxidant and antimicrobial activities.

Methods: The dry distillates were prepared by dry distillation method from the *Acacia seyal* stem, stem wood and stem bark and investigated chemically by GC-MS analysis.

Results: The percentage yield of dry distillates (*Dokhan*) wer found to be 4.0 %, 4.8%, 1.4.1% v/w for stem, stem wood and stem bark respectively. GC-MS analysis revealed the presence of one hundred and twenty three constituents in the stem distillate with major constituents of solerone (7.27%), furfural (7.15%), catechol (7.11%), syringol (5.56%), allo-inositol (4.86%), mequinol (4.81%), furfuralcohol (3.35%), 3-methyl-1,2-cyclopentanedione (3.24%), phenol (2.73%), homovanillyl alcohol (2.56%) and 3-cresol (2.11%). the wood distillate show detection of eighty compounds, with main compounds: 1, 3-dimethyl-5-methoxypyrazol (10.61%), syringol (6.75%), furfuralcohol (5.24%), mequinol (4.49%), 1,2-anhydro-3,4,5,6-alloinositol (4.26%), 3-methyl-1,2-cyclopentanedione (3.42%), catechol (3.37%), 3-methoxycatechol (3.22%), homovanillyl alcohol (2.78%), homosyringic acid (2.40%), 3-cresol (2.18%), 3-methyl-2-cyclopentenone (2.44%) and 1,2-cyclopentanedione (2.03%). Sixty six compounds were detected in the distillate bark and the main compounds were found to be hexadecanoic (62.83%), catechol (3.38%), tetrapentacontane (3.18%), phenol (2.72%), mequinol (2.30%) and 2-ethylfurane (2.22%).

Conclusion: The result concludes that the medicinal Sudanese *Dokhan* have good potential as sources of different bioactive compounds and antioxidants.

Keywords: Chemical composition, Dry distillates, *Acacia*, *seyal*, Stem, Stem wood, Stem bark

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INTRODUCTION

Acacia seyal (Fabaceae) is a small to a medium important tree in Sudan, locally known as *Talih.* The pleasantly fragrant fumigate of the stem or stem wood, known as *Dokhan* is widely used by Sudanese women as an aromatic and medicinal plant for cleanness and perfume purposes. *Dokhan* from the stem or stem wood was known for its potent antimicrobial and antioxidant activity]. The aromatic oil from the plant traditionally used by Sudanese women showed preservative and therapeutic properties in addition to its pleasant aroma [1-7].

The Fabaceae family produces more nitrogen-containing secondary metabolites than other plant families such as quinolizidine, pyrrolizidine, indolizidine, piperidine, pyridine, pyrrolidine and many other nitrogenous compounds [8]. *Acacia* genus was reported to have many secondary metabolites such as amines, alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, amino acids, essential oils, diterpenes, phytosterol, triterpenes, saponins and hydrolyzable tannins. The most evident and best known are polysaccharides (gums) and complex phenolic substances (condensed tannins) [9]. *A. seyal* is highly nitrogen-fixing and moderately salt tolerant species and characterized by high content of proteins, phenols and flavonoids [10].

The present paper represents the first attempt to investigate and compare of the content and composition of the dry distillates (*Dokhan*) of *A. seyal* stem, stem wood and stem bark with potent antioxidant and antimicrobial activities [1] which is traditionally used by Sudanese women for cosmetic and therapeutic purposes mainly for the treatment of candidiasis, genital yeast infection, urinary tract infection, severe stomach cramps, diarrhea, vomiting, respiratory tract disease, cold and throat infection, wound and skin infection and toothache [1, 11].

MATERIALS AND METHODS

Plant materials collection and preparation

The stem of *A. seyal* was collected from Omdurman local market, Sudan, and then it was authenticated by taxonomist at the Department of Silviculture, Faculty of Forestry, University of Khartoum. The voucher specimen; IKR4, December-2015 was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Science and Technology. The collected material was cleaned, dried and separated into two parts: one part of the plant material was chopped into small pieces and the other was separated into stem bark and stem wood and they were preserved separately for further studies.

Phytochemical screening

The phytochemical constituents of the plant material were detected using a standard procedure described by Farhat *et al.*, *et al.*, [12], Prashant *et al.*, [13] and Mosa *et al.*, [14]. The physiochemical parameters of the plant materials were detected according to the methods described by the WHO [(15].

Distillates preparation and determination of physiochemical properties

The stem, stem wood and stem bark distillates were prepared from the samples by dry distillation technique described by lewandowki and Milchert [16] with a minor modification. The percentage yield

was determined in (v/w) and (w/w) with reference to the dried sample weight.

Solubility, specific gravity, refractive index, acid value, ester value and a saponification value of the prepared distillates were determined according to the British Pharmacopoeia, (2002) [17, 18].

GC-MS analysis

The gas chromatography-Mass spectrometry analysis was carried out on gas chromatograph coupled to a mass spectrometer (GC-MS QP). The temperature was programmed at 180 ℃ for 2 min. at a rate of 10c/min, and then increased to 289 ℃ for 1 min. at a rate of 15c/min and the dry distillate was injected with split injection mode. The identification of different components was achieved from their mass spectra, retention time (RT), compared to those in NIST library [19]. The fragmentation mode of major constituents was carried out and their m/z value was compared with those obtained in the Mass spectrometry spectra.

RESULTS

Phytochemical screening

The phytochemical screening of *A. seyal* stem, stem wood and stem bark (table 1) revealed the presence of tannuns, terpenoids, cardiac glycoside and reducing sugar in the three assessed materials. Flavonoids, alkaloids and steroids are detected in the stem and stem wood, whereas, they are absence in the stem bark. Saponnins are not detected in any of the three plant materials.

Percentage yields and physiochemical properties of the distillates

The oily dry distillates of *A. seyal* stem, stem wood and stem bark were found to be slightly different in their physiochemical properties. The physiochemical property of stem bark is more differ from that of stem and stem wood (table 2).

Table 1: Qualitative phytochemical screening of *A. seyal* stem, stem wood and stem bark

Phytochemical	Result		
	Stem	Wood	Bark
Flavoniods	+ve	+ve	-ve
Alkaloids	+ve	+ve	-ve
Tannins	+ve	+ve	+ve
Saponins	-ve	-ve	-ve
Steroids	+ve	+ve	-ve
Terpenoids	+ve	+ve	+ve
Cardiac glycosides	+ve	+ve	+ve
Reducing sugars	+ve	+ve	+ve

+: presence,-: absence

Table 2: Percentage yields and physiochemical properties of dry distillates of *A. seyal* stem, wood and stem bark

Plant part	Percentage yield		Physiochemical				
	V/W	W/W	Sp. Gr.	Ref. I	Ac. V.	Es. V	Sap. V
Stem	15 %	83.8 %	0.95g	1.341	23.6 ml/g	34.8 ml/g	50.9 ml/g
Stem wood	14.1 %	48 %	0.95g	1.352	24.6 ml/g	34.8 ml/g	58.9 ml/g
Stem bark	13.6 %	53 %	0.95g	1.341	21.7 ml/g	16.1 ml/g	37.8 ml/g

V/M= volume/weight, W/W= weight/weight, Sp. Gr.= specific gravity, Ref. I = refractive index, Ac. V= acid value, Es. V. = ester value, Sap. V. = saponification value

GC-MS analysis

The results of GC-MS analysis of the distillates showed a slight difference between the chemical constituents of stem and stem wood distillates, whereas, the chemical constituent of stem bark distillate is more different from those two distillates. The analysis revealed the presence of one hundred and twenty-three, eighty and sixty-six constituents of the stem, stem wood and stem bark distillates respectively (table 3 and fig. 1, 2, 3, 4).

Table 3: GC-MS result of *A. seyal* stem, stem wood and stem bark dry distillates

Compound number	R. T	Compound name	Area %		
			Stem	Wood	Bark
1	3.034	Propanal	-	0.85	-
2	3.090	Propanoic acid	-	-	0.11
3	3.090	Butanoic acid	-	0.85	0.24
4	3.090	Quinone	-	-	0.07
5	3.100	Pyridine,3-methyl	-	-	0.23
6	3.102	Furfural	7.15	0.22	-
7	3.236	Methylthiirane	0.13	-	-
8	3.317	Furfuralcohol	3.35	5.24	1.47
9	3.449	Unknown	0.76	-	-
10	3.581	Aniline	-	-	0.47
11	3.581	(S)-5-Hydroxymethyl-2-(5H)-furanone	-	0.07	-
12	3.628	4-Methylhexanoic acid	0.12	-	-
13	3.662	Avitrol	-	-	0.18
14	3.662	2,3-Pentanediol	-	0.35	-
15	3.836	2-(Tetrahydrofuran-2-yloxy)-ethanol	0.11	0.52	-
16	3.915	dl-Threonine	0.64	-	-
17	3.991	2-Ethylfurane	1.15	1.75	2.22

18	4.041	1, 3-Dimethyl-5-methoxypyrazol	-	10.61	-
19	4.045	2-Acetylfuran	0.66	0.73	0.46
20	4.088	Dumasin	1.51	0.97	-
21	4.134	2-Cyclohexenol	0.13	-	-
22	4.209	1,2-cyclopentanedione	1.28	2.03	0.15
23	4.245	β-Octalactone	0.12	0.38	-
24	4.332	3,5-Lutidine	0.14	-	0.10
25	4.334	Angelica lactone	-	0.42	0.08
26	4.340	4-Methyl-2(5H)-furanone	-	-	0.10
27	4.350	L-Leucine,ethyl ester	-	0.45	0.35
28	4.365	2-Cyclohexenone	0.09	0.16	0.79
29	4.427	6-Methyl-2,2-diphenyl-cyclohexanone	0.42	0.30	-
30	4.533	Suberone	0.23	-	0.42
31	4.640	Valerolactone	0.08	-	-
32	4.694	Ethylene dipropionate	0.42	-	-
33	4.740	γ-Ethoxybutyrolactone	0.16	-	-
34	4.770	5-methylfurfural	0.46	-	-
35	4.805	3-Methyl-2-cyclopentenone	1.03	2.44	1.07
36	4.874	Octanamide	-	0.43	-
37	4.893	Hexanoic acid	0.08	1.42	-
38	4.936	Methyl 2-furoate	0.09	-	0.06
39	4.975	Phenol	2.73	1.79	2.72
40	5.088	Maleamic acid	0.12	0.72	-
41	5.137	2-Hydroxy-γbutyrolactone	0.44	-	-
42	5.221	3,4-dimethylcyclopent-2-en-1-one	0.21	-	0.41
43	5.231	γ-Crotolactone	-	1.50	0.28
44	5.289	Decylamide	0.82	-	-
45	5.328	2,3-Dimethylpyrazine	-	-	0.04
46	5.329	3,5-Dimethyl-2(5H)-furanone	0.51	0.42	0.22
47	5.400	Tetrahydro, furfuryl alcohol	0.81	0.89	0.43
48	5.515	Unknown	-	-	0.27
49	5.515	Uridine	0.33	0.15	
50	5.651	4-methyl-4-Hepten-3-ol	0.27	-	-
51	5.724	3-Methyl-1,2-cyclopentanedione	3.24	3.42	0.58
52	5.864	2,3,4,5-Tetramethylfuran	0.12	-	0.06
53	5.865	Methyl 3-butenoate	-	-	0.17
54	5.890	Dihydro-2-methyl-3-furanone	-	-	0.13
55	5.905	3-Ethylpyridine	-	-	0.06
56	5.927	2,3-dimethyl-2-cyclopentenone	0.99	0.73	0.14
57	6.002	4-Methyl-2(5H)-furanone	0.22	0.34	-
58	6.103	Orthocresol	0.98	1.66	0.75
59	6.150	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	0.30	0.85	0.36
60	6.226	2-Acetylpyrrole	0.09	-	0.06
61	6.281	2-Hexyltetrahydrofuran	0.45	-	-
62	6.361	Heptanoic acid	0.21	0.12	-
63	6.414	3-Cresol	2.11	2.18	1.50
64	6.466	Thymine	-	0.20	-
65	6.489	Octanal	0.32	-	-
66	6.598	γ-tridecalactone	0.06	1.43	-
67	6.640	15-Amino-1-pentanol, N,O-diacetyl-	0.09	-	-
68	6.707	Mequinol	4.81	4.49	2.30
69	6.783	2-Octenal	1.56	-	-
70	6.876	4-Pyridinol	1.31		0.65
71	6.951	1-(2-furyl)pyrrole	-	1.12	-
72	6.954	4-Octyne	-	-	0.19
73	6.960	2,6-Dimethylphenol	0.22	-	-
74	7.064	Maltol	0.53	0.51	0.25
75	7.153	2-Isopropyl-2,5-dimethyl-cyclohexanone	0.95	-	-
76	7.316	2-Ethyl-6-methylpyridine	-	-	0.12
77	7.382	Cyclohexane	0.23	-	-
78	7.449	2-Octyne	-	0.21	0.07
79	7.571	p-Xylenol	0.92	0.57	0.20
80	7.685	Diethyl azodicarboxylate	0.28	-	-
81	7.758	Benzoic acid	0.12	-	-
82	7.832	Octanoic acid	0.46	0.07	-
83	7.866	Meobal	0.56	-	-
84	8.031	Phenylethyl Alcohol	0.08	-	0.07
85	8.088	Creosol	0.24	1.92	0.43
86	8.230	Tetrahydro-2-Furancarboxylic acid	0.92	0.44	-
87	8.332	Catechol	7.11	3.37	3.38
88	8.567	3-Hexenedioic acid, trans-	1.15	1.36	0.53
89	8.625	methyl salicylate	0.07	-	-
90	8.783	Thiophene,2-propyl	0.24	-	-
91	8.849	DL-Lactide	0.53	-	-

92	8.945	Camphor	0.20	-	-
93	9.065	Di-n-butyl diazene,	-	1.70	
94	9.123	Phorone	0.09	-	-
95	9.172	Threitol, acetylated	0.09	-	-
96	9.282	2-Methyl hydroquinone	0.57	0.71	-
97	9.356	3-Methoxycatechol	1.80	3.22	0.23
98	9.440	Hydroquinone	1.03	0.90	0.49
99	9.600	4-Ethylguaiacol	1.21	1.20	0.25
100	9.703	Orcinol	1.56	1.23	0.45
101	9.789	Solerone	7.27	-	0.12
102	9.876	Lactide	0.20	-	-
103	10.121	4-Vinylguaiacol	0.68	0.75	0.58
104	10.249	4-Butoxy-1-butanol	0.23	-	-
105	10.345	L-Glutamine	-	0.59	-
106	10.484	4-Methylcatechol	0.87	-	0.56
107	10.558	Propylthiophene	0.19	-	-
108	10.646	Syringol	5.56	6.75	0.83
109	10.678	2,3-Xylenol	-	0.54	0.13
110	10.740	Eugenol	0.14	0.26	-
111	10.796	3,4-Dimethoxyphenol	032	-	-
112	10.876	Dihydroeugenol	0.26	0.22	-
113	10.975	3,7,11,15-Tetramethyl-1-hexadecyn-3-ol	0.08	-	-
114	11.052	4-Ethylcatechol	0.68	0.49	-
115	11.092	(E)-Dodec-2-enyl ethyl carbonate	-	0.46	-
116	11.092	Unknown	-	-	0.50
117	11.233	2,3,5-Trimethylphenol	0.07	-	-
118	11.285	4-Methoxythiophenol	0.14	-	-
119	11.357	Vanillin	0.43	0.58	-
120	11.462	Isoeugenol	0.16	1.02	-
121	11.527	Sinenofuranol	0.18	-	-
122	11.626	1-Decyne	0.27	0.72	-
123	11.965	Homovanillyl alcohol	2.56	2.78	0.08
124	12.027	Trans-Isoeugenol	0.91	-	-
125	12.544	Allo-Inositol	4.86	0.15	-
126	13.017	5-tert-Butylpyrogallol	1.71	1.71	-
127	13.121	4-vinylsyringol	1.10	1.04	0.17
128	13.216	1,2-Anhydro-3,4,5,6-alloinositol	0.84	4.26	0.08
129	13.347	Dodecanoic acid	1.09	-	-
130	13.557	4-vinyl-2,6-dimethoxyphenol	1.34	0.19	-
131	13.857	Amol	0.05	-	-
132	13.935	Unknown	0.48	-	-
133	14.106	3-Hydroxy-4-methoxycinnamic acid	0.40	-	-
134	14.204	Senkyunolide	0.32	-	-
135	14.544	3-sulfanylheptanal	0.21	-	-
136	14.746	2,4-Dimethoxyphenol	-	0.50	-
137	14.910	Methoxyeugenol	0.71	0.37	-
138	15.095	Syringaldehyde;	0.38	0.37	-
139	15195	(Z)-p-Methoxy-cinnamic acid	-	1.32	-
140	15.390	3,4,5-Trimethoxyacetophenone	0.20	-	-
141	15.599	Heptadecanoic acid	0.06	-	-
142	15.713	Unknown	1.14	-	-
143	15.980	Unknown	0.16	-	-
144	16.225	Xanthoxylin	0.81	1.1	-
145	16.382	Tetradecanoic acid	0.66	-	-
146	16.684	Cerulignol	-	0.19	-
147	16.721	Homosyringic acid	1.80	2.40	-
148	16.806	Nonadecanol	0.05	-	-
149	17.314	Unknown	0.50	-	-
150	17.399	Unknown	0.19	-	-
151	17.828	Cymol	0.27	-	-
152	18.146	2,4-Dimethoxybenzyl alcohol	0.12	-	-
153	18.284	Lidocaine	0.18	-	-
154	18.337	Hexadecanoic acid	0.09	0.93	-
155	18.451	2-Imidazolidinethione	-	-	0.89
156	18.700	Unknown	1.57	-	-
157	28.922	Plastoquinone 3	-	0.28	0.31
158	29.193	Tetrapentacontane	-	-	3.18
159	31.051	Hexadecanoic acid, hexadecyl ester	-	-	62.83
160	31.606	Unknown	-	-	2.31
Total	-	-	100	100	100

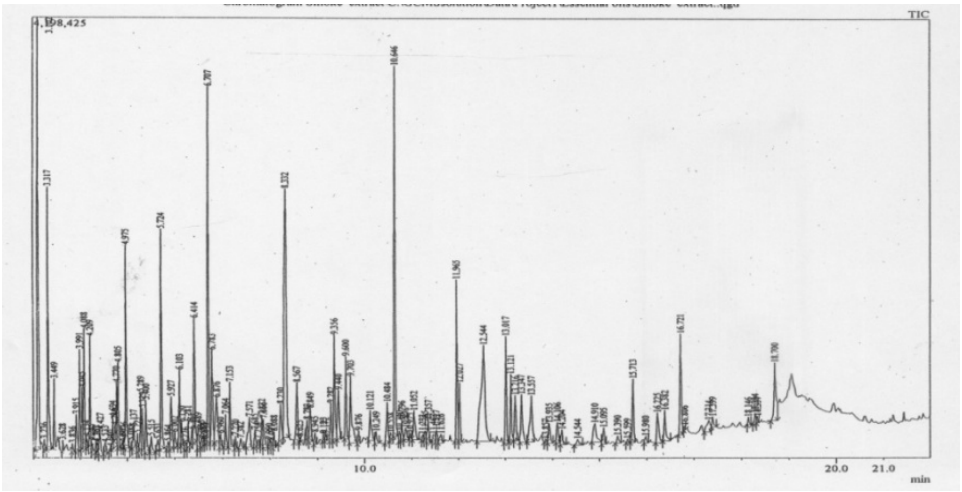

Fig. 1: GC-MS chromatogram of *A. seyal* stem dry distillate

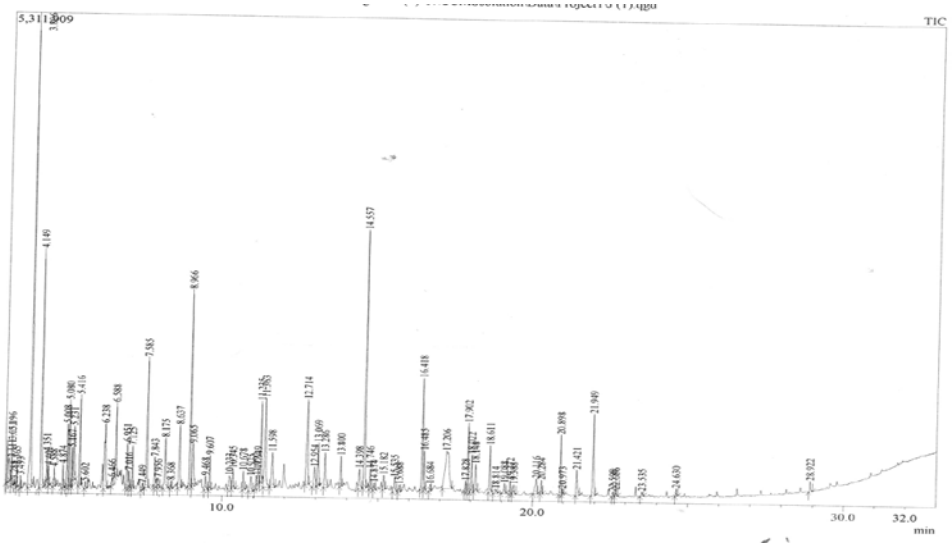

Fig. 2: GC-MS chromatogram of *A. seyal* stem wood dry distillate

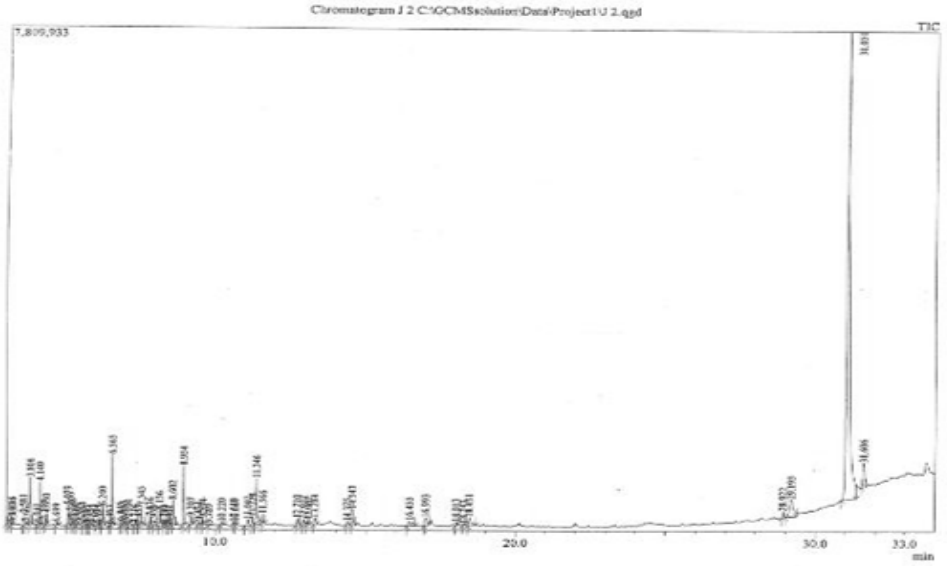

Fig. 3: GC-MS chromatogram of *A. seyal* stem bark dry distillate

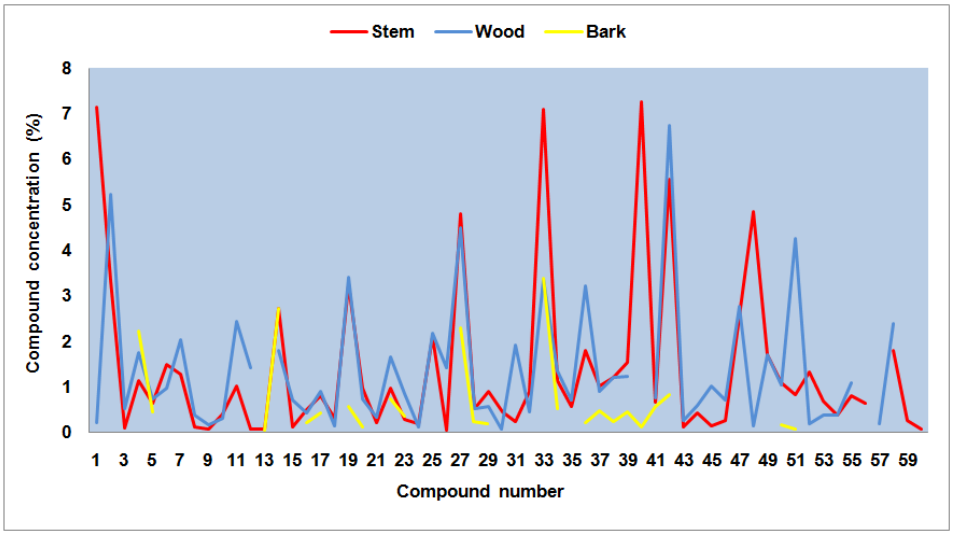

Fig. 4: The common and major compounds in the dry distillates of *A. seyal* stem, stem wood and stem bark 1-59 = compounds number (table 3): 6,8,15,17,19,20,22,23,28,29,35,37,38,39,40,46,47,49,51,56,57,58,59,62,63,66,68,74,79,82,85,86,87,88,96,97,98,99,100,101, 103,108,110,119,120,122,123,125,126,127,128,130,137,138,144,145,146,147,148

DISCUSSION

The various phytochemical compounds found in the plants are known to have beneficial importance in industrial and medical sciences [20]. The preliminary phytochemical analysis of *A. seyal* stem, stem wood and stem bark (table 1) revealed the presence of tannins, terpenoids, cardiac glycoside and reducing sugar in all assessed plant materials. Flavonoids, alkaloids and steroids are detected in the stem and stem wood, whereas, they are absence in the stem bark. Saponnins are not detected in any of the three plant materials. The results showed that the stem and stem wood parts which are traditionally used by Sudanese women [1] have the same secondary metabolites, whereas, the stem bark which is slightly used showed different in secondary metabolites. The presence of flavonoids, alkaloids, tannins, steroid, terpeniods, cardiac glycosides and reducing sugars are compatible with the secondary metabolites of Fabaceae family [21]. These findings are reported for the first time about the secondary metabolites of *A. seyal* stem, wood and bark.

The diverse health benefit of the plants is usually known to be through the numerous phytochemicals extractions [22]. The dry distillation of *A. seyal* stem and stem wood is well known for its medicinal and cosmetic values among the Sudanese women [3]. The dry distillates of *A. seyal* stem, stem wood and stem bark were found to be oily pale brown to red in color, with specific aromatic odor and water soluble. The distillates yields (table 2) were; 15 %; 14.1%; 13.6% (v/w) and 83.87%; 48%; 53% (w/w) of the stem, stem wood and bark, respectively. Their specific gravity; refractive index; acid value; saponification value and ester value (table 2) were found to be (0.95g, 0.95, 0.95); (1.341, 1.352, 1.341); (23.6 ml/g, 24.6 ml/g, 21.7 ml/g); (50.9 ml/g, 58.9 ml/g, 37.8 ml/g) and (34.8 ml/g, 34.3 ml/g, 16.1 ml/g) in the stem, stem wood and bark respectively. The dry distillates of *A. seyal* stem and stem wood mainly used by Sudanese women [3] were found to be slightly different in their physiochemical properties, whereas, the physiochemical property of the slightly used distillate of stem bark is more different from that of stem and stem wood (table 2). These findings are reported for the first time about the percentage content and physiochemical properties of *A. seyal* dry distillates.

Regarding the GC-MS analysis, the difference between the chemical constituents of stem and stem wood distillates had been slightly, while, that of stem bark distillate was more difference from these two distillates (fig. 1, 2, 3, 4 and table 3). The identification of the constituents was done by the direct comparison of their retention times; peak areas; molecular weight; formula and fragmentation patterns according to the NIST library [19].

The analysis revealed the presence of many nitrogenous compounds which was compatible with the chemistry of the Fabaceae [20]. The dry distillate from the stem represented the presence of one hundred and twenty three constituents of which one hundred and seventeen of them have been identified, and the major constituents were found to be solerone (7.27%), furfural (7.15%), catechol (7.11%), syringol (5.56%), allo-inositol (4.86%), mequinol (4.81%), furfur alcohol (3.35%), 3-methyl-1,2-cyclopentanedione (3.24%), phenol (2.73%), homovanillyl alcohol (2.56%) and 3-cresol (2.11%). the stem wood dry distillate was composed of eighty compounds, all of them have been identified with major compounds of 1, 3-dimethyl-5-methoxypyrazol (10.61%), syringol (6.75%), furfur alcohol (5.24%), mequinol (4.49%), 1,2-anhydro-3,4,5,6-alloinositol (4.26%), 3-methyl-1,2-cyclopentanedione (3.42%), catechol (3.37%), 3-methoxycatechol (3.22%), homovanillyl alcohol (2.78%), homosyringic acid (2.40%), 3-cresol (2.18%), 3-methyl-2-cyclopentenone (2.44%) and 1,2-cyclopentanedione (2.03%). Sixty six compounds were detected in the dry distillate of the stem bark of which sixty-three have been identified and the main compounds were found to be hexadecanoic (62.83%), catechol (3.38%), tetrapentacontane (3.18%), phenol (2.72%), mequinol (2.30%) and 2-ethylfurane (2.22%). Some of the detected and identified compounds could be artefacts due to the process of dry distillation.

The main and common constituents found in the three distillates (fig. 4) were found to be proportional to their antioxidant and antimicrobial activity [1]. It is noteworthy to point out the relationship between these main constituents known of their antimicrobial and antioxidant properties and their content in the dry distillates. The different concentrations of these active constituents and their proportions in the three dry distillates explain clearly the potent antimicrobial activity of the stem [1] and its application in the fumigation traditions by Sudanese women. These findings about the chemical composition of *A. seyal* stem, stem wood and stem bark dry distillates are reported for the first time and adds to the current literature with regard to the presence of many nitrogenous compounds in the Fabaceae family [8] and abundance of essential oils, diterpenes, phytosterols and triterpenes in the *Acacia* genus.

CONCLUSION

These research conclude that the medicinal Sudanese *Dokhan* which is a fumigation process done by Sudanese women have good potential as sources of different bioactive compounds and antioxidants.

It is noteworthy to add that the chemical composition and content of detected components in the three dry distillates of the plant were

proportional and comply with their uses in Sudanese traditional medicine. Sudanese women usually use stem wood for *Dokhan*, sometimes they use the stem and rarely the stem bark.

AUTHORS CONTRIBUTION

Design of the work was done by the first and second authors Dr. Ikram Mohamed Eltayeb and Dr. Itmad Awad Elhassan. Interpretation of data, writing and correction of the manuscript was done by the first author Dr. Ikram Mohamed Eltayeb. The experimental part of the work was done by Ms. Jihad Hasab Elrasoul and Ms. Eiman Salah Eldind.

CONFLICT OF INTERESTS

Declared none

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