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Comparative Studies of Antioxidant and Antimicrobial Activities of *Acacia seyal* Stem, Stem Wood and Stem Bark Dry Distillates

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ABSTRACT

Acacia seyal is well known in the Sudanese traditional medicine for its therapeutic value. The pleasantly fragrant fumigate bath, locally known as *Dokhan*, mainly from the stem or stem wood of the plant is widely used for cosmetic and medicinal purposes. The investigation and a composition of antioxidant and antimicrobial activities of the dry distillates (*Dokhan*) prepared by dry distillation method from the stem, stem wood and stem bark is the objective of the present study. The results showed remarkable antioxidant and antimicrobial activities of the dry distillates. The antioxidant activity assayed by using DPPH radical scavenging technique was found to be 94±0.01%, 95±0.03, 93±0.02 for the stem, its wood and bark respectively compared to Propylgallate, standard antioxidant agent 90±0.01. The disc diffusion procedure was used for antimicrobial activity assessment which revealed the highest activity in the stem wood dry distillate with inhibitory zone diameter ranging from 20 to 36 mm.

Keywords: Comparative study, Antioxidant, Antimicrobial, *Acacia*, *seyal*, Stem, Stem wood, Stem bark, Dry Distillates

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INTRODUCTION

Acacia seyal, known as *Talih* in Sudan is a medium tree of the family Fabaceae with important uses in Sudanese traditional medicine. The stem is widely used to treat fungal infections, mainly genital yeast infections and as chewing sticks with antimicrobial activity. A bark decoction is used against leprosy and dysentery; as stimulant and purgative agent; as aphrodisiac with cytotoxic activity; as a pharmaceutical constituent in making emulsions and torches and as a masking agent for bitter substances [1-4]. A root decoction mixed with leaves of *Combretum glutinosum* and curdled milk causes strong dieresis [4].

The aromatic oil from the plant traditionally used by Sudanese women showed preservative and therapeutic effects of beneficial properties. The pleasantly fragrant fumigate of stem or stem wood locally known as *Dokhan*, is widely used for its cosmetic, aromatic and therapeutic value. It is used mainly for the treatment of vaginal and urinary tract infections, curing body aches, restoration after child birth and to relieve rheumatic pain [4, 5].

Vaginal yeast infections and urinary tract infections are very common in women and caused by *Candida albicans* [6, 7]. The urinary tract infections are polymicrobial aerobic and anaerobic. The predominant aerobes are *Staphylococcus aureus* [8, 9] and commonest anaerobes are Gram-negative *bacilli* and *cocci* mainly *E. coli*[10]. More than 90% urinary tract infections are caused by *E. coli* bacterium [11]and *Pseudomonas aeruginosa* was reported as one agent of UTIs [12].

The present paper represents the first attempt to investigate and compare of the antioxidant and antimicrobial activities of the dry distillates (*Dokhan*) of *A. seyal* stem, stem wood and stem bark traditionally used by Sudanese women for therapeutic and cosmetic purposes.

MATERIALS AND METHOD

Dry Distillates Preparation

The stem of *A. seyal* was collected from Omdurman local market, Sudan and identified at the department of siliviculture, Faculty of Forestry, University of Khartoum and the voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Science and Technology. One part of the plant material was chopped into small pieces and the other part was separated into stem bark and stem wood and used separately for dry distillation. The dry distillates of stem, stem wood and stem bark were prepared from the samples by dry distillation technique described by lewandowki and Milchert [13] with a minor modification.

Antioxidant Activity Test

The antioxidant activity of the oily distillate was assayed by the free radical scavenging method described by Mensor *et al*.,[14] and Kexue *et al*.,[15]. In the assay, 10 µl from the dry distillates (5mg/ml) were added to 90µl of 300µM DPPH solution placed in a 96-well microtiter plate. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. The mixture was incubated in the dark at room temperature for 30 min. After incubation, the absorbance of the remaining DPPH was read against a blank sample at 517 nm using multiplate reader spectrophotometer. Propylgallate was used as the positive control and DMSO as the negative control. All tests and analyses were carried out in triplicate. The inhibition of free-radical DPPH in percent (%) or the capacity to scavenging the DPPH radical (radical scavenging activity) was expressed as EC_{50} value (mg ml-1), and calculated by the following equation:

Scavenging activity (%) = (A control – A sample)/ A control ×100

Antimicrobial Activity Test

The antimicrobial activity of the distillate was carried out using disc diffusion technique as described by the National Committee for Clinical Laboratory Standards Guidelines. The organism suspension was diluted with sterile physiological solution to 10^8 cfµ/ml (turbidity= McFarland standard 0.5). One hundred micro liters of suspension were swabbed uniformly on the surface of Mueller Hinton agar (MHA) and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (whatman No. 1.6 mm in diameter) were placed on the surface of the MHA agar and soaked with 20ul of a solution of each dry distillate (50ml). The inoculated plates were incubated at 37^0c for 24 hours in the inverted position and inhibition zones diameters were measured [16].

Determination of Minimum Inhibitory Concentration (MIC)

The MICs values were determined by agar disc diffusion method and the plates were prepared into series diluted concentrations of the dry distillates [16].

Dry Distillates Analysis

The three prepared dry distillates were analyzed by gas chromatograph coupled to a mass spectrometer (GC-MS QP). The temperature was programmed at 180^0C for 2 min. at rate of 10c/min, and then increased to 289^0C for 1 min. at rate of 15c/min, the dry distillate was injected with split injection mode. The identification of different components was achieved from their mass spectra and retention time (RT), compared to those in NIST library [17]. The fragmentation pattern of major constituents was carried out and their m/z value was compared with those obtained in the Mass spectra.

RESULTS AND DISCUSSION

The antioxidant activity results of the oily prepared dry distillates of *A. seyal* stem, stem wood and stem bark, assessed by DPPH radical scavenging assay were shown in table 1 and figure 1. The three dry distillates showed potent antioxidant activity, higher than the standard antioxidant in addition to the highest activity exerted by the wood dry distillate. The activity was found to be 94±0.01%, 95±0.03, 93±0.02 for the stem, its wood and bark, respectively compared to the standard antioxidant agent of 90±0.01. These finding were reported for the first time about the antioxidant activity of the plant dry distillates. The result was compatible with the current literature about the antioxidant activity of the volatile compounds [18, 19]. *The antioxidant activity* may be due to the medicinal effects attributed to the plant distillates *and* justified the traditional use of the stem, stem wood and stem bark in the fumigation process traditionally known as *Dohkan*. The wood dry distillate possessed activity higher than the stem and stem bark dry distillates while the stem activity was higher than that of its bark. These findings could explain the frequent use of stem wood than the stem itself or its bark for fumigation in the *Dokhan* process by Sudanese women.

Table 1: Antioxidant activity result of *A. seyal* Stem, Stem wood and Stem bark oily Dry Distillates

Tested Sample	% of DPPH free radical activity (±SEM) (RSA%±SD)
Stem distillate	94±0.01
Stem wood distillate	95±0.03
Stem bark distillate	93+-0.02
Propylgallate	90±0.03

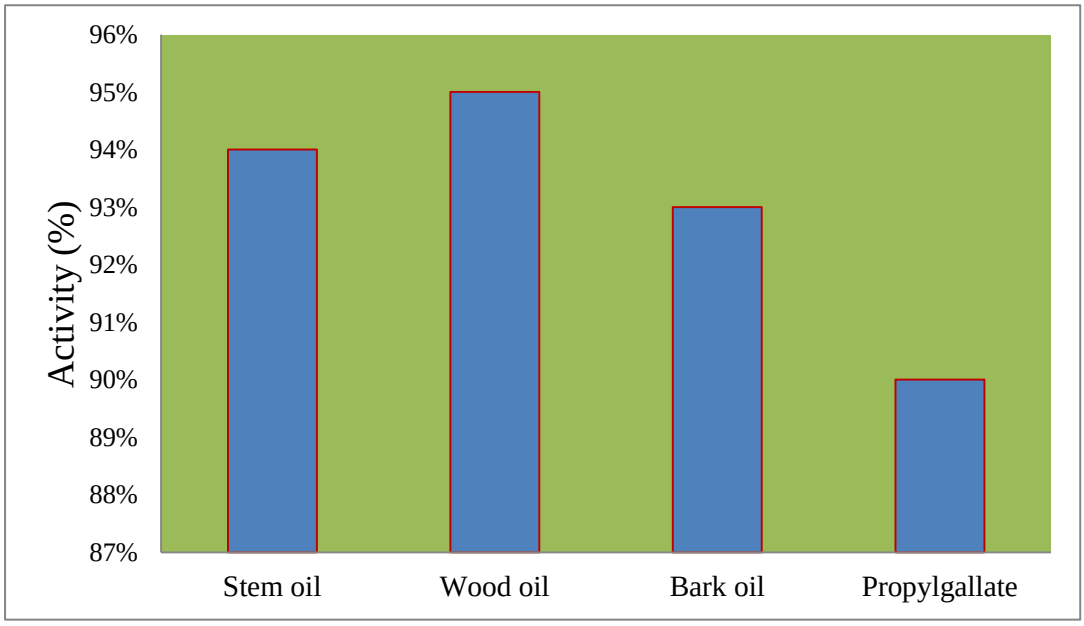

Figure 1: Antioxidant activity of *A. seyal* Stem, Stem wood and Stem bark oily Dry Distillates

The results of antimicrobial activity of oily dry distillates against four bacteria and two fungi were shown in table 2 and figure 2. The three dry distillates showed remarkable antimicrobial activity against the six tested microorganisms in addition to the highest activity exerted by the wood dry distillate. The stem wood dry distillate was found to posses activity higher than the stem and bark dry distillates, and stem dry distillate higher than bark dry distillate. The inhibition zones against the six organisms were found to be in the range of 36-20mm; 25-15mm; 20-13mm in the dry distillates of stem wood, stem and stem bark, respectively. The MIC ranges were 12.5–6.25µl for stem and stem wood dry distillates and 12.5 µl for bark dry distillate.

The results showed that, the oily distillate was highly active against *Candida albican*s, which is the principal causative agent of the candidiasis, a fungal yeast infection that affects the skin, genitals, throat, mouth and blood [20]. This result could justify the traditional use of *Dokhan* in treating of genital yeast infections.

S. aureus was reported to be the predominant aerobes agent of UTIs infections [8, 9] bacilli (*B. subtilis*) and cocci (*E. coli*) which are the commonest anaerobes agents [(10)]. More than 90% urinary tract infections caused by *E. coli* [11]. *P. aeruginosa was reported as one agent of* UTIs [1)]. A wide range of infections, from relatively minor skin infections to more serious infections of lungs were reported to be caused by *S. aureus*. It is one of the commonest bacterial agents in the skin and soft tissue infections [21, 22], wounds infectious[23], and for the respiratory infections[24]. It is a human bacterial pathogen causing dental caries and periodontal disease [25, 26]. *C. albicans* and *A. niger* were reported to cause superficial fungal infections of skin [22]. *A. niger* is one of the agents to cause respiratory tract infection[22]; *E. coli* is the main agent responsible for diarrhea[27, 28]. *P. aeruginosa* is an organism responsible for opportunistic infections, such as respiratory tract inflammations [(28)] and was reported to be one of the commonest bacterial agents in the skin and soft tissue infections [21, 22]. The promising results of activity against these organisms could justify the traditional use of the plant *Dokhan* for the treatment of urinary tract infections, severe stomach cramps, diarrhea, vomiting, respiratory tract and throat infection, toothache, wound and skin infections. These findings about the antimicrobial activity of the plant dry distillates are reported for the first time and could be a useful contribution to the current literature of the antimicrobial activity of the volatile oils [30- 32]

It is noteworthy to add that the antioxidant and antimicrobial activities of 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.

Table 2: Antimicrobial activity results of *A. seyal* Stem, Stem wood and Stem bark oily Dry Distillate

Tested Organisms	Inhibitory zones diameters (IZD)			MIC Value		
	Stem	Wood	Bark	Stem	Wood	Bark
Candida albicans	20 mm	20 mm	14 mm	6.25 µl	6.25 µl	12.5 µl
Aspergillus niger	22 mm	35 mm	20 mm	6.25 µl	6.25 µl	12.5 µl
Staphylococcus aureus	15 mm	25 mm	15 mm	12.5 µl	12.5 µl	12.5 µl
Escherichia coli	18 mm	28 mm	15 mm	6.25 µl	12.5 µl	12.5 µl
Pseudomonas aeruginosa	19 mm	25 mm	13 mm	6.25 µl	6.25 µl	12.5 µl
Bacilli subtilis	25 mm	36 mm	15 mm	12.5 µl	12.5 µl	12.5 µl

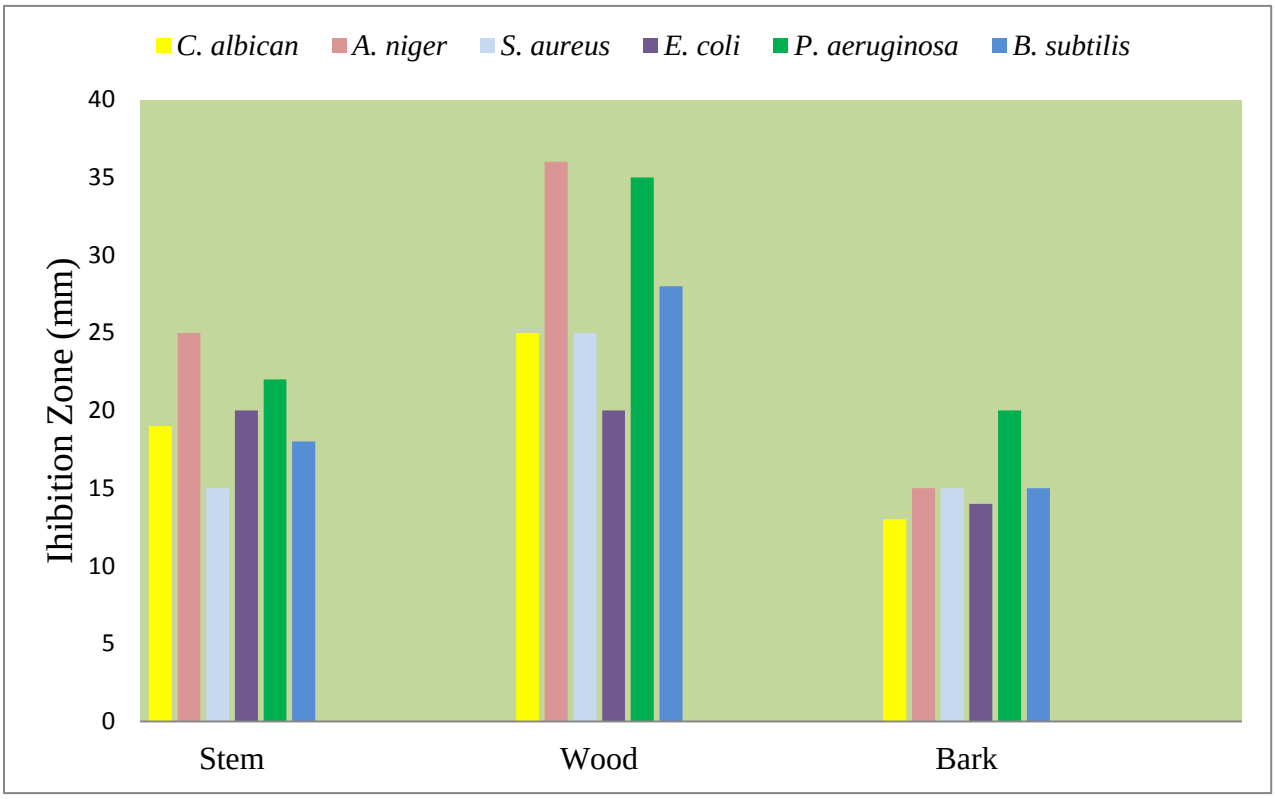

Figure 2: The Antimicrobial activity of *A. seyal* stem, Stem wood and Stem bark oily Dry Distillates

Analysis of the three dry distillates using GC-MS technique revealed the presence of one hundred and twenty two; eighty and sixty three compounds in the dry distillates of the stem, stem wood and stem bark respectively. The main constituents found in the three distillates were shown in table 3 and figure 3. 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 and its application in the fumigation traditions by Sudanese women.

Table 3: The Common and Major Compounds in the Dry Distillates of *A. seyal* Stem, Stem wood and Stem bark

Peak NO.	R.T	Formula	Compound Name	Area %		
				Stem	Wood	Bark
1	3.102	$C_5H_4O_2$	Furfural	7.15	0.22	-
2	3.317	$C_5H_6O_2$	Furfuralcohol	3.35	5.24	1.47
3	3.836	$C_6H_{12}O_3$	2-(Tetrahydrofuran-2-yloxy)-ethanol	0.11	0.52	-
4	3.991	C_6H_8O	2-Ethylfurane	1.15	1.75	2.22
5	4.045	$C_6H_6O_2$	2-Acetylfuran	0.66	0.73	0.46
6	4.088	C_5H_8O	Dumasin	1.51	0.97	
7	4.209	$C_5H_6O_2$	1,2-cyclopentanedione	1.28	2.03	0.15
8	4.245	$C_8H_{14}O_2$	β-Octalactone	0.12	0.38	
9	4.365	C_6H_8O	2-Cyclohexenone	0.09	0.16	0.79
10	4.427	$C_{19}H_{20}O$	6-Methyl-2,2-diphenyl-cyclohexanone	0.42	0.30	-
11	4.805	C_6H_8O	3-Methyl-2-cyclopentenone	1.03	2.44	1.07
12	4.893	$C_6H_{12}O_2$	Hexanoic acid	0.08	1.42	-
13	4.936	$C_6H_6O_3$	Methyl 2-furoate	0.09		0.06
14	4.975	C_6H_6O	Phenol	2.73	1.79	2.72
15	5.088	$C_4H_5NO_3$	Maleamic acid	0.12	0.72	
16	5.329	$C_6H_8O_2$	3,5-Dimethyl-2(5H)-furanone	0.51	0.42	0.22
17	5.400	$C_5H_{10}O_2$	Tetrahydro, furfuryl alcohol	0.81	0.89	0.43
18	5.515	$C_9H_{12}N_2O_6$	Uridine	0.33	0.15	-
19	5.724	$C_6H_8O_2$	3-Methyl-1,2-cyclopentanedione	3.24	3.42	0.58
20	5.927	$C_7H_{10}O$	2,3-dimethyl-2-cyclopentenone	0.99	0.73	0.14
21	6.002	$C_5H_6O_2$	4-Methyl-2(5H)-furanone	0.22	0.34	-
22	6.103	C_7H_8O	Orthocresol	0.98	1.66	0.75
23	6.150	$C_7H_{10}O_2$	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	0.30	0.85	0.36
24	6.361	$C_7H_{14}O_2$	Heptanoic acid	0.21	0.12	-
25	6.414	C_7H_8O	3-Cresol	2.11	2.18	1.50
26	6.598	$C_{13}H_{24}O_2$	γ-tridecalactone	0.06	1.43	-
27	6.707	$C_7H_8O_2$	Mequinol	4.81	4.49	2.30
28	7.064	$C_6H_6O_3$	Maltol	0.53	0.51	0.25
29	7.571	$C_8H_{10}O$	p-Xylenol	0.92	0.57	0.20
30	7.832	$C_8H_{16}O_2$	Octanoic acid	0.46	0.07	-
31	8.088	$C_8H_{10}O_2$	Creosol	0.24	1.92	0.43
32	8.230	$C_5H_8O_3$	Tetrahydro-2-Furancarboxylic acid	0.92	0.44	-
33	8.332	$C_6H_6O_2$	Catechol	7.11	3.37	3.38
34	8.567	$C_6H_8O_4$	3-Hexenedioic acid, trans-	1.15	1.36	0.53
35	9.282	$C_7H_8O_2$	2-Methyl hydroquinone	0.57	0.71	-
36	9.356	$C_7H_8O_3$	3-Methoxycatechol	1.80	3.22	0.23
37	9.440	$C_6H_6O_2$	Hydroquinone	1.03	0.90	0.49
38	9.600	$C_9H_{12}O_2$	4-Ethylguaiacol	1.21	1.20	0.25
39	9.703	$C_7H_8O_2$	Orcinol	1.56	1.23	0.45
40	9.789	$C_6H_8O_3$	Solerone	7.27	-	0.12
41	10.121	$C_9H_{10}O_2$	4-Vinylguaiacol	0.68	0.75	0.58
42	10.646	$C_8H_{10}O_3$	Syringol	5.56	6.75	0.83
43	10.740	$C_{10}H_{12}O_2$	Eugenol	0.14	0.26	-

44	11.357	$C_8H_8O_3$	Vanillin	0.43	0.58	-
45	11.462	$C_{10}H_{12}O_2$	Isoeugenol	0.16	1.02	-
46	11.626	$C_{10}H_{18}$	1-Decyne	0.27	0.72	-
47	11.965	$C_9H_{12}O_3$	Homovanillyl alcohol	2.56	2.78	0.08
48	12.544	$C_6H_{12}O_6$	Allo-Inositol	4.86	0.15	-
49	13.017	$C_{10}H_{14}O_3$	5-tert-Butylpyrogallol	1.71	1.71	-
50	13.121	$C_{10}H_{12}O_3$	4-vinylsyringol	1.10	1.04	0.17
51	13.216	$C_6H_{10}O_5$	1,2-Anhydro-3,4,5,6-alloinositol	0.84	4.26	0.08
52	13.557	$C_{10}H_{12}O_3$	4-vinyl-2,6-dimethoxyphenol	1.34	0.19	-
53	14.910	$C_{11}H_{14}O_3$	Methoxyeugenol	0.71	0.37	-
54	15.095	$C_9H_{10}O_4$	Syringaldehyde	0.38	0.37	-
55	16.225	$C_{10}H_{12}O_4$	Xanthoxylin	0.81	1.1	-
56	16.382	$C_{14}H_{28}O_2$	Tetradecanoic acid	0.66	-	-
57	16.684	$C_{10}H_{14}O_2$	Cerulignol	-	0.19	-
58	16.721	$C_{10}H_{12}O_5$	Homosyringic acid	1.80	2.40	-
59	16.806	$C_{19}H_{40}O$	Nonadecanol	0.05	-	-
60	17.828	$C_{10}H_{14}$	Cymol	0.27	-	-
61	18.146	$C_9H_{12}O_3$	2,4-Dimethoxybenzyl alcohol	0.12	-	-
62	18.337	$C_{17}H_{34}O_2$	Hexadecanoic acid	0.09	0.93	-

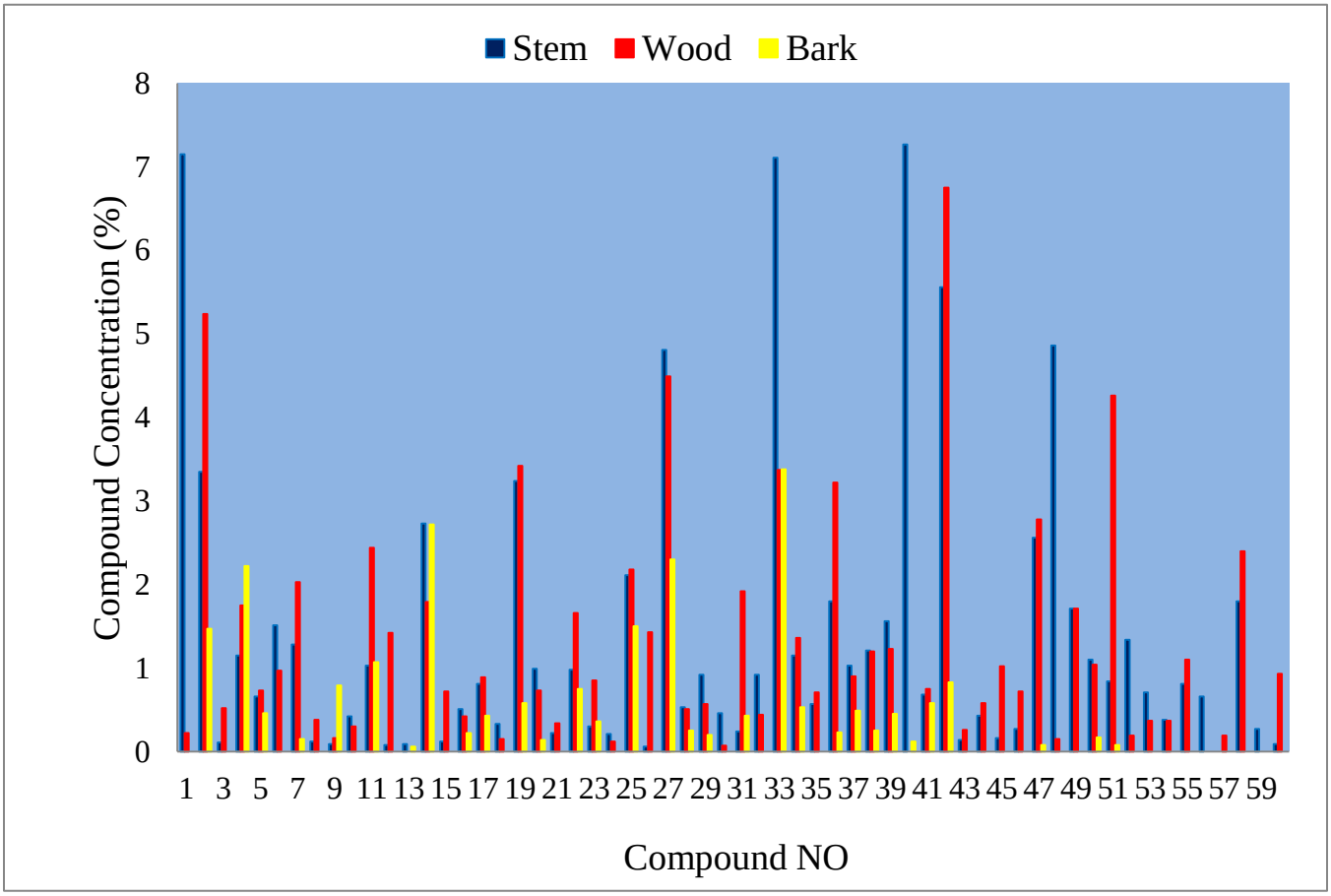

Figure 3: The Common and Major Compounds in the Dry Distillates of *A. seyal* stem, Stem wood and Stem bark

CONCLUSION

The present study concluded that, *A. seyal* stem and its wood and bark dry distillates posses potent antioxidant and antimicrobial activities that justify the traditional use of the stem and its wood in Sudanese traditional medicine for the treatment of candidiasis, genital yeast infection, urinary tract infection, severe stomach cramps, diarrhea and vomiting, respiratory tract disease, cold and throat infection, wound and skin infection and toothache. Several components of the dry distillates are known of their antimicrobial and antioxidant activities which gave solid grounds to support the claims of their uses in Sudanese traditional medicine.

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