

Isolation, Identification of Some Chemical Constituents and Antimicrobial Activity of Different Extracts from *Withania somnifera* Growing at Albaha Region, KSA

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The purpose of this study is to investigate some chemical constituents and biological activities of different extracts of *Withania somnifera* (WS) growing in Albaha region KSA. The volatile oils of both leaves and stems were extracted by hydrodistillation method and analyzed using Gas Chromatography/ Mass spectrometry (GC/MS) technique. The pet ether extracts of different parts (leaves, stems and seeds) of the plant were fractionated into acetone insoluble fraction, unsaponifiable matter and fatty acids which were identified using (GC/MS) analysis. All defatted plant powder of each organ were extracted with methanol (80 %) which subsequently fractionated using (chloroform, ethyl acetate and n-butanol) respectively. Flavonoidal content of all extracts was determined using the standard Aluminum chloride spectrophotometric method while phenolic content was determined by using Folin-Ciocalteu spectrophotometric method. The results of the antimicrobial activity of different showed that, the extracts of leaves and stems only showed activity against gram positive bacteria while, all gram negative bacteria are resistant to all extracts except ethyl acetate extract of leaves.

Keywords: Antimicrobial Activity; Folin-Ciocalteu; GC/MS; Lipid Constituents; Volatile Oils; *Withania somnifera*.

The plant natural products plays an important role in treating human diseases. Whereas human beings since ancient times interested to study and isolated active compounds from plants¹. *Withania somnifera* has been an important medicine plant belonging to *Solanaceae* family. It can be found naturally distributed in many areas on the world mostly the drier regions, subtropical zones of the Mediterranean region and northern

Africa to south west Asia. *W. somnifera* has been known for its ability to promote public health and mental health over the years without toxicity or side effects^{2,3}. Several groups of chemical constituents have been extracted, isolated and identified from this plant such as steroidal lactones, alkaloids, flavonoids, tannins, saponins, reducing sugars and terpenoids⁴⁻⁶. Active constituents are present in this plant which explains its medicinal

properties such as (Tropin, Anaferine, Anahygrine and Withanine) besides withanolides (withaferin A and withanolide D)⁷. *W. somnifera* also considered as a good source for fatty acids⁸. Many researchers documented the biological and pharmaceutical properties of *W. somnifera* and proved its activity as antimicrobial, anti-inflammatory, antioxidant, antiulcer, antidepressant, used in treatment of various kinds of cancers such as colon, lung, skin, blood and breast cancer and cardio protective^{9,10}. Additionally, the plant has a great potential in treatment of burns, wounds and dermatological diseases, have a beneficial effects in preventing brain and neurological disorders such as anxiety, Alzheimer, Parkinson and Schizophrenia¹¹⁻¹⁴.

By reviewing the available literature, there is no data about the lipid constituents of the plant so, the present study has focused to investigate some chemical constituents and antimicrobial activity for different extracts of leaves, stems and seeds of *W. somnifera*.

MATERIALS AND METHODS

Plant Material

WS plant was collected from Albaha region, Saudi Arabia during December 2018. The plant parts leaves, stems and seed were separated. All parts were air dried for two weeks. subsequently, ground each part to get a fine powder.

Extraction of volatile oil

About 180 g of plant powder (leaves and stems) of *WS* were subjected to hydrodistillation method¹⁵ using Clevenger apparatus for about 4 hours. The obtained volatile oils were analyzed using GC/MS analysis with the following conditions: about 200 mg of each oil were transferred to a glass test tube 15ml, mixed with 1 ml methanol, vortexed for 2 min, and filtered through 0.22 μ m Nylon membrane. A portion from the clear extract was transferred to auto sampler vial, and a volume of 1.5 μ l was injected. instrument: Clarus 500 GC/MS Perkin Elmer model. The oven was programmed as follows: Initial temperature was 43 °C (hold 5 min) to 65 °C (rate 8 °C/min, hold 3.0 min), to 100 °C (rate 4 °C/min, hold 8.5 min), to 280 °C (rate 15 °C/min / hold 2 min). The injector temperature was 265 °C, and the injection volume was 1.5 μ L, and the split ratio was 50:1. Samples were acquired by applying a total MS scan from 40 to 500 m/z (500

scan/sec). NIST 2008 was used to characterize the eluted compounds.

Extraction of Lipid Constituents

About 500 g of powdered material (leaves, stems and seeds) of *WS* were extracted with Petroleum ether (40-60 °C) using Soxhlet apparatus. The pet. ether extract passed over fuller's earth to remove the colored pigments in case of leaves and stems extracts, after then dehydrated by passing on an anhydrous sodium sulfate, filtered and evaporated under vacuo at 40 °C. Boiling acetone was added to the pet. ether residues, separately, for leaves and stems only and left overnight. The formed white precipitate (acetone insoluble fraction) was filtered off. The acetone soluble fraction of leaves, stems and pet. ether extracts of seeds were saponified by refluxing for 5 hours with alcoholic KOH. The alcoholic solution for each part was concentrated and diluted with cold distilled water. Then, the unsaponifiable matter was extracted by shaking with chloroform (three times). In addition, the aqueous sap solution of leaves, stems and seeds were acidified and extracted several times with chloroform to extract the fatty acids which were dissolved in methanol containing HCl. Each mixture refluxed for 4 hrs., concentrated, extracted with chloroform and evaporated to obtain the fatty acid methyl esters. All lipid constituents were subjected to GC/MS analysis. which were performed using the following conditions: Apparatus: Perkin Elmer Clarus 600 gas chromatograph linked to a mass spectrometer. An aliquot of the extract was injected into the Elite-5MS column of 30 m, 0.25 mm film thickness, 0.25 mm internal Diameter capillary column using the following temperature program. The GC/MS system starts with the initial oven temperature of 40°C, holds for 2 min, then increases to 200°C with a 5°C min⁻¹ rate of increase and holds for 2 min. From 200°C, the temperature increases at a rate of 5°C min⁻¹ to 300°C and holds for 2 min. The injector temperature was maintained at 280°C. The interface temperature was 240°C and the source temperature was 220°C. The vacuum of the system was maintained at 1.1e5 and the electron energy was 70 eV. Helium was used as a mobile phase at a 10 ml/min flow rate. Mass spectral detection was performed in electron ionization mode by scanning at 40–600 m/z. Finally, unknown compounds were identified by comparing the spectra with those

recorded in the National Institute of Standard and Technology (2005) and WILEY (2006) libraries (Coates 2000; Linstrom and Mallard, 2005). The total time required for analyzing a single sample was 58 min.

Fractionation of Methanolic Extracts

The defatted powdered material (leaves, stems and seeds) of *WS* were macerated in aqueous methanol (80%) for three days with continuous stirring and filtered. The combined alcoholic extracts were evaporated under reduced pressure at 45 °C and hot distilled water was added to the alcoholic extract for each part. Partition the aqueous filtrate for each part with chloroform, ethyl acetate and butanol sequentially.

Determination of Total Phenolics and Flavonoidal Content

The total phenolic content (TPC) of different extracts of *WS* leaves and stems (Butanol BE-1, BE-2, Chloroform CE-1, CE-2, Ethyl acetate EE-1, EE-2 and Methanol ME-1, ME-2) were determined by using Folin-Ciocalteu spectrophotometric method¹⁶. Total flavonoidal content (TFC) of different extracts of *WS* leaves and stems were determined by using the standard Aluminium chloride spectrophotometric method¹⁷.

Determination of Antimicrobial Activity

The antimicrobial activity of different extracts was performed at Albaha regional research laboratory, Albaha, Saudi Arabia. It carried out against two type of gram positive bacteria (*Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212)), two gram negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and one fungal strain *Candida albicans* (ATCC 10231). Susceptibility screening test was carried out by disc diffusion method¹⁸. Amoxicillin and Fluconazole were used as standard drugs.

RESULTS AND DISCUSSION

Volatile Oils of *WS* Leaves and Stems

The volatile oil of both leaves and stems was obtained as a pale yellow oil with characteristic odor which was analyzed using GC/MS as in figure (1 and table 1). The data revealed that, both oils are consist of a mixture of thirty six and thirty seven compounds respectively with 9-Octadecenamide as a major compound (42.81% and 44.49%

respectively). The alcoholic compounds present in leaves are more than in stems. Hydrocarbons were present relatively high in stems more than leaves. As well as aromatics, sesquiterpenes, diterpenes, ketones and aldehydes were present in a small percentage in both. Two ester compounds namely methyl palmitate and isooctyl laurate were present in volatile oil of leaves, while only one ester (isooctyl laurate) was exist in volatile oil of stems. Sulfur compounds were present in volatile oil for stems only as butyl octadecyl sulfite and octadecyl 2-pentenyl sulfite. By reviewing the available literature, it was found that these data were reported for the first time for this plant.

Investigation of The Lipid Constituents

The GC/MS analysis of acetone insoluble fraction of leaves and stems indicated the presence of three n-hydrocarbons compounds with n-Pentacosane (n-C₂₅H₅₂) as major component in leaves while, in stems there are six compounds in which nonacosane (n-C₂₉H₆₀) was major component. Besides one fatty alcohol compound was present in acetone insoluble fraction of stems which identified as: 1-Pentacontanol (n-C₂₅H₅₀O). By reviewing the available literature, it was found that these data were reported for the first time for this plant. The results are summarized in table (2).

The obtained results from the GC/MS analysis of the unsaponifiable matter in leaves, stems and seeds present in table (3) unsaponifiable matter constituents were varying between hydrocarbons, alcohols, aldehydes and ketones. For leaves the data showed the presence of hydrocarbons as the majority (50.14 %). Also, the other (43.66 %) distributed among alcohols and fatty alcohols with 3,7,11,15-tetramethyl-2-hexadecenol in a high percentage (34.57%). Also, two ketone compounds (5.04 %). One aldehydic compound was present with small percentage (1.16 %) which is 15-heptadecenal. These data were in agreement with that reported by Abdelgawad et al., in 2015¹⁹ where they investigated the unsaponifiable matter from the petroleum ether extract of *W. somnifera* leaves growing in Egypt by GC/MS. They proved the presence of 2-ethyl hexanol, hexadecane and heptacosane but disagree with our results in the percentages of these compounds. Furthermore, The results of unsaponifiable matter of stems led to identification of fifteen compounds in which the hydrocarbons are in large quantities

with the majority for (tetracontane 25.97%). Also, alcoholic compounds were present in percentage of 32.09 %. In addition, there was only one ketone compound in percentage 7.20 %. The data showed the presence of one aldehydic compound in small quantity (hexanal 1.17 %). Two steroidal

Table 1. GC/MS data for volatile oil of *WS* leaves and stems

No.	Compound	R _t (min)	PercentagesM ⁺		B.P.	Fragments	
			Leaves	Stems			
1	Benzene	2.77	—	0.52	78	78	26(5), 39(15), 51(22)
2	Cyclohexane	2.86	2.35	1.39	84	56	41(55), 69(30), 84(82)
2	Pentanal	3.10	0.71	—	86	44	29(42), 41(30), 58(48)
3	2-Methylbutanal	2.73	—	0.21	86	41	27(42), 29(95), 39(35)
4	Toluene	4.71	0.31	—	92	91	39(15), 65(12), 92(55)
5	2-Ethylfuran	3.30	0.98	—	96	81	29(10), 53(45), 67(15)
6	Methyl cyclohexane	3.87	—	0.24	98	83	27(16), 39(18), 41(40)
7	Heptane	3.46	—	0.78	100	43	15(10), 29(50), 57(50)
8	3-Methylhexane	3.07	1.26	0.70	100	43	29(25), 57(55), 71(59)
9	Hexanal	5.55	0.27	0.37	100	44	29(35), 41(67), 56(82)
10	3-Hexen-1-ol	7.55	1.74	—	100	67	29(15), 41(75), 55(38)
11	3-Ethyl-2,2-dimethyloxirane	9.45	0.94	—	100	59	31(40), 41(57), 85(22)
12	2-Methyl- 2-pentanol	3.95	—	0.27	102	59	29(10), 41(22), 45(50)
13	2-Methylheptane	5.06	0.23	0.21	114	43	29(18), 57(86), 70(20)
14	3-Methylheptane	5.30	0.49	0.39	114	43	29(40), 41(55), 57(72)
15	4-Hydroxy-4-methyl- 2-pentanone	6.83	0.40	0.37	116	43	39(10), 59(35), 83(5)
16	2-Pentylfuran	2.90	—	0.47	138	81	27(10), 41(10), 53(18)
17	2,5-Dimethyl-3,4-hexadiol	10.20	1.80	0.41	146	73	43(25), 55(20), 85(5)
18	4-allyloxy-2-methyl pentan-2-ol	9.55	3.14	0.93	158	43	41(30), 45(20), 59(44)
19	2,2,4,6,6-pentamethylheptane	13.41	4.05	3.41	170	57	29(15), 41(25), 71(10)
20	2,2,4,4-tetramethyloctane	14.96	0.54	0.38	170	57	43(18), 70(4), 99(18)
21	Tridecane	22.19	0.34	0.24	184	57	29(23), 43(90), 71(50)
22	1-Dodecanol	31.39	0.49	0.52	186	55	29(37), 43(97), 69(87)
23	Octyl cyclohexane	32.25	0.38	1.39	196	83	29(8), 41(21), 55(45)
24	2-Methyltridecane	31.67	0.23	0.21	198	57	29(18), 43(98), 71(55)
25	1-Tridecanol	33.99	0.78	0.79	200	55	29(20), 41(65), 69(82)
26	2,5-bis(1,1-dimethylethyl) phenol	33.01	0.66	0.89	206	191	29(5), 41(10), 57(17)
27	2,6,10-Trimethyl dodecane	34.15	0.63	0.68	212	57	29(20), 43(48), 71(70)
28	Hexadecane	34.10	2.93	2.21	226	57	29(17), 43(74), 71(61)
29	1-Hexadecanol	34.58	2.58	2.29	242	55	30(22), 41(77), 69(82)
30	2-Ethyl-2-methyl tridecanol	35.79	0.96	0.98	242	57	43(50), 71(80), 83(60)
31	3-Methylheptadecane	35.53	0.54	0.59	254	57	29(15), 43(50), 71(60)
32	n-Heptadecanol	36.17	2.85	2.79	256	55	29(18), 43(57), 69(79)
33	1-Nonadecene	35.66	2.58	14.06	266	57	29(38), 43(92), 69(82)
34	Nonadecane	35.73	3.39	4.78	268	57	29(23), 43(80), 71(65)
35	Methyl palmitate	36.51	2.67	—	270	74	29(10), 43(35), 55(32)
36	9-Octadecenamamide	41.30	42.81	44.49	281	59	29(10), 41(22), 72(50)
37	2,6,10,14-Tetramethylhexadecane	38.35	1.51	1.48	282	57	29(18), 43(61), 71(70)
38	1-Eicosanol	38.70	4.09	1.25	289	43	29(32), 57(85), 69(75)
39	1-Docosene	37.51	4.30	5.39	308	57	29(30), 43(95), 69(63)
40	Isooctyl laurate	38.06	3.30	2.08	312	43	57(85), 71(62), 85(55)
41	Heptacosane	37.15	2.77	—	380	57	29(19), 43(92), 71(61)
42	Butyl octadecyl sulfite	36.91	—	0.75	390	57	43(55), 71(45), 85(35)
43	Octadecyl 2- pentanyl sulfite	37.61	—	1.09	404	71	43(60), 57(55), 85(45)
			100	100			

R_t = Retention time, M⁺ = Molecular ion peak, B.P. = Base peak

compounds were present in the unsaponifiable matter of the seeds which were identified as ergost-5-en-3-ol (64.45%) and stigmasterol (12.06%) with one triterpenoidal compound α - amyirin (6.94 %) besides the presence of two hydrocarbon compounds (15.25 %). The data showed presence of one fatty alcohol compound 3,7,11,15-Tetramethyl-2-hexadecenol (1.30 %) which was also present in the unsaponifiable matter for leaves and stems. These findings were

compatible with the recent study by Mishra and Patnaik in 2020²⁰ on methanolic extract of whole plant where the GC/MS results showed the presence of this compound also.

The GC/MS analysis after methylation of fatty acids of leaves, stems and seeds indicated the presence a variety of saturated and unsaturated fatty acids. The overall results are summarized in table (4). Fatty acids which exist in leaves were varying between saturated and unsaturated where,

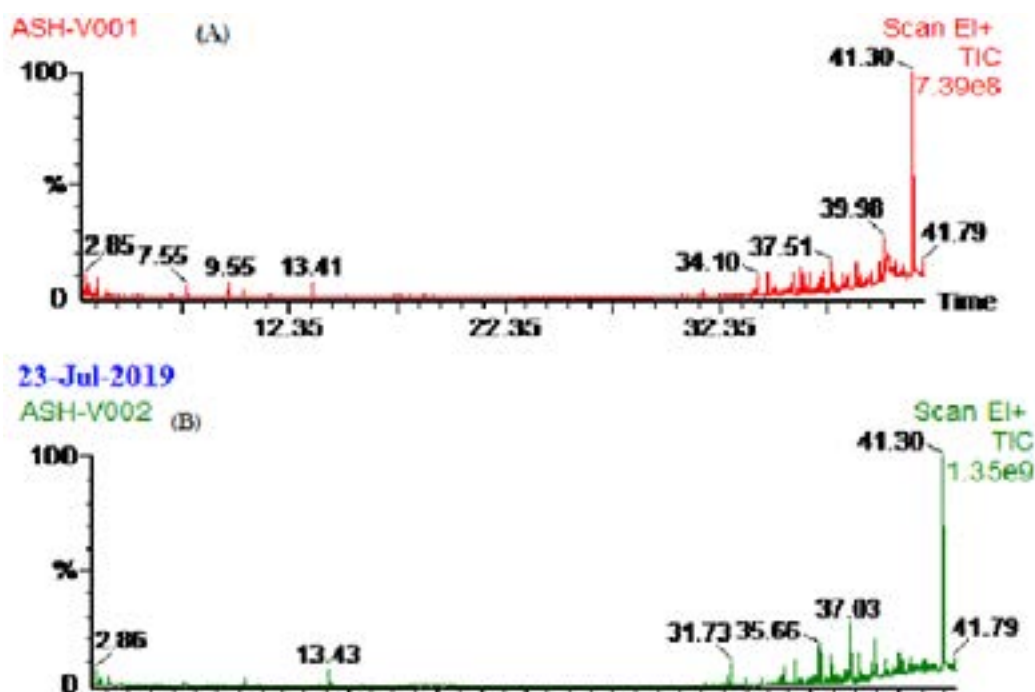


Fig. 1. GC-MS chromatogram of volatile oils of WS leaves(A) and stems(B)

Table 2. GC/MS data for acetone insoluble fractions of leaves and stems

No.	Compound	R _t (min)	Percentages		M.W.
			Leaves	Stems	
1	Pentacosane	31.46	84.84	—	352
2	Heptacosane	30.63	6.25	2.390	380
3	Nonacosane	24.55	—	75.36	408
4	Triacotane	24.18	—	0.790	422
5	Dotriacotane	26.05	—	3.950	450
6	Pentatriacontane	25.43	—	9.790	492
7	Hexatriacontane	29.93	8.91	—	507
8	Tetracontane	23.00	—	5.440	563
9	1-Pentacontanol	25.16	—	2.280	719
			100	100	

R_t = Retention time, M.W.= Molecular weight

the unsaturated fatty acids were predominate (84.97 %) in which monounsaturated fatty acids with oleic acid as a major compound (76 %). Polyunsaturated fatty acid (11,14,17-Eicosatrienoic acid 6.64 %) and diunsaturated fatty acids were present in a small quantity (linoleic acid 2.33 %). Moreover, the saturated fatty acids were represented by four fatty acids. Palmitic acid had the highest percentage (10.45 %). These data were in accordance with that reported by Chatterjee et al., in 2010⁸ where, they found that, the hexane extract of leaves was rich with oleic, palmitic and linoleic acids. Furthermore, it was found that, the stems were rich with saturated fatty acids (63.77 %) and the majority for palmitic acid (29.95 %). Also, the unsaturated fatty acids were present in stems as (17.06 %), most of them were diunsaturated with

linoleic acid forming (15.67 %). In addition, a small amount from monounsaturated was present in percentage (1.39 %) as one compound which was identified as elaidic acid. The results of stems were similar with that stated by Namdev and Gupta in 2015²¹ where they studied methanolic extract of *W. somnifera* stems and the presence of palmitic, margaric, linoleic and arachidic acids but the percentages of these compounds were differ from our results. Finally, the seeds were found to contain a high percentage of monounsaturated fatty acids which constitutes about (64.59 %) with oleic acid as the main compound (63.78 %). Saturated fatty acids also present in seeds with (35.41%) and 10,13-dimethyltetradecanoic acid as predominant (33.85%). The lowest percentage was for palmitic acid (0.06 %). Some fatty acids which present in

Table 3. GC/MS data of unsaponifiable matter of *W*/leaves, stems and seeds

No.	Compounds	R _t (min)	Percentages			M.W.
			Leaves	Stems	Seeds	
1	Heptane	3.15	1.87	—	—	100
2	Hexanal	4.76	—	1.17	—	100
3	2-Methyl-2-pentanol	3.75	2.02	—	—	102
4	3-Methyl-3-hexanol	5.56	1.22	—	—	116
5	4-Methoxy-4-methyl-2-pentanone	6.68	1.87	—	—	130
6	2-Ethyl-1-hexanol	8.80	2.78	7.46	—	130
7	4-Isopropyl-3-(2-propenyl) cyclohexanol	24.10	—	2.43	—	182
8	1-Dodecanol	9.53	—	0.74	—	186
9	1-Pentadecene	24.14	2.69	—	—	210
10	Hexadecane	24.75	34.27	—	0.80	226
11	E-15-Heptadecenal	20.38	1.16	—	—	252
12	6,10,14-Trimethyl-2-pentadecanone	21.00	3.17	7.20	—	268
13	1-Octadecanol	21.50	—	3.69	—	270
14	3,7,11,15-Tetramethylhexadecane	25.81	—	0.56	—	282
15	1-Nonadecanol	22.61	1.19	—	—	284
16	3,7,11,15-Tetramethyl-2-hexadecanol	23.87	34.57	6.17	1.30	296
17	4,8,12,16-Tetramethylheptadecane	26.06	2.65	2.16	—	296
18	1-Eicosanol	27.10	—	7.84	—	298
19	1-Docosanol	30.28	1.88	—	—	326
20	Heptacosane	27.16	2.28	11.67	—	380
21	Ergost-5-en-3-ol	27.44	—	—	64.45	400
22	Stigmasterol	28	—	—	12.06	412
23	Triacotane	26.33	2.68	—	—	422
24	Alpha amyirin	29.28	—	—	6.94	426
25	Nonacosanol	25.42	—	3.76	—	424
26	Dotriacotane	27.96	1.78	4.96	14.45	450
27	Hexatriacontane	28.75	1.92	—	—	507
28	Tetracontane	30.00	—	25.97	—	563
29	Tetratetracontane	31.18	—	14.22	—	619
			100	100		

seeds were similar to that the study conducted by Arora et al., in 2018²², whereas, the percentages of myristic acid and palmitic acid in our result were less than the percentages which described in their results. Oleic acid was present in our result as a major compound.

Total Phenolics and Flavonoidal Content

The results of TPC and TFC for all the extracts are showed in figure (3). As is clear from

the results, the butanol extract of stems (BE-2) exhibited the highest TPC as 215 mg of GAE/g powder weight. followed by ethyl acetate extract of leaves (EE-1) with 213.4 mg of GAE /g of powder weight. On the other hand, the methanol extract of leaves (ME-1) exhibited the lowest TPC of 92.6 mg of GAE /g. These results disagree with that stated by Pal et al., in 2015²³ as they investigated the TPC of *WS* leaves extract. Their results revealed

Table 4. GC/MS data of fatty acids methyl ester of *WS* leaves, stems and seeds

No.	Compounds	R _t (min)	Percentages			M.W.
			Leaves	Stems	Seeds	
1	Methyl Phthalate	27.40	—	19.17	—	180
2	Methyl 7- methyl nonanoate	28.79	—	2.25	—	186
3	Methyl 8-methyldecanoate	26.50	—	1.69	—	200
4	Methyl myristate	19.56	2.07	4.34	0.16	242
5	Methyl Palmitoleate	14.78	—	—	0.81	268
6	Methyl Palmitate	21.90	10.45	29.95	0.06	270
7	Methyl 10,13- dimethyl Tetradecanoate	15.09	—	—	33.85	270
8	Methyl Margarate	22.93	—	1.35	—	284
9	Methyl Linoleate	23.61	2.33	15.67	—	294
10	Methyl Oleate	28.63	76	—	63.78	296
11	Methyl Elaidate	20.60	—	1.39	—	296
12	Methyl Stearate	23.90	—	10.07	—	298
13	Methyl Isopropyl Palmitate	22.87	0.96	1.59	—	312
14	Methyl 11,14,17-Eicosatrienoate	23.68	6.64	—	—	320
15	Methyl Arachidate	25.67	1.55	5.68	0.79	326
16	Methyl Behenate	27.30	—	4.23	0.31	354
17	Methyl Lignocerate	21.78	—	—	0.24	382
			100	100		

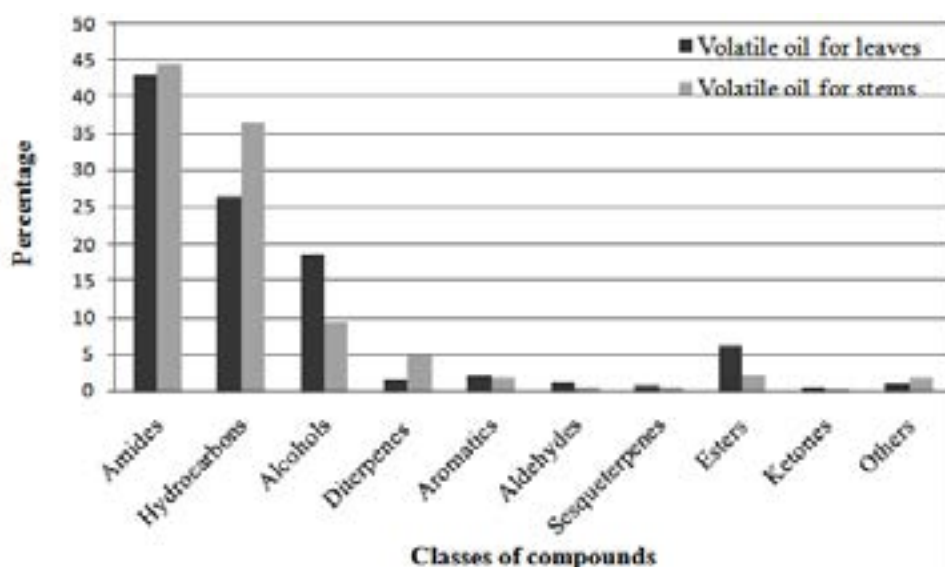


Fig. 2. Percentage of different classes of compounds in volatile oils of *WS* leaves and stems

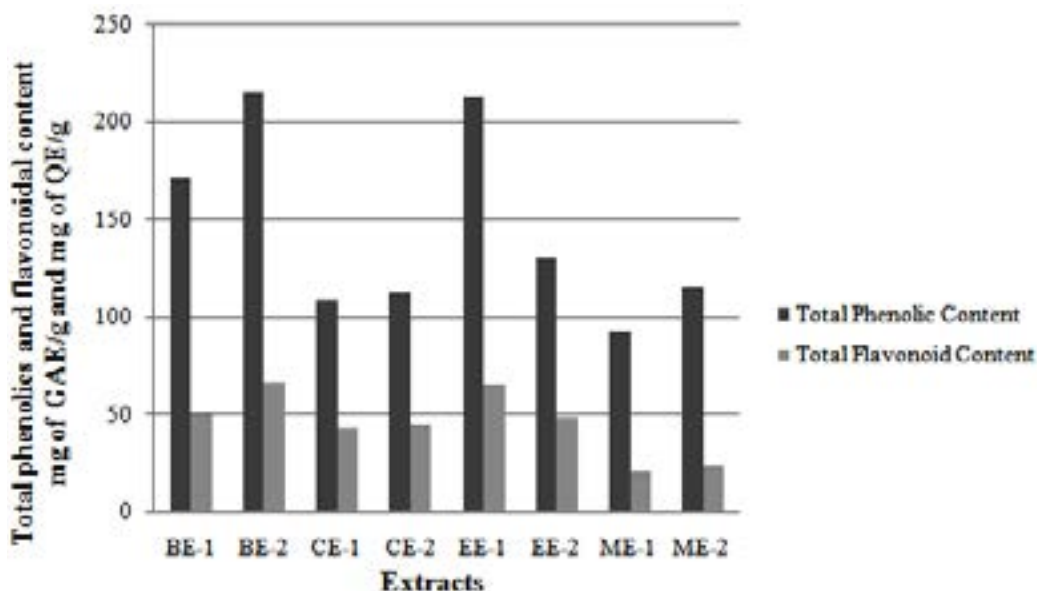


Fig. 3. Total Phenolics and Flavonoidal content of WS different extract

Table 5. Antimicrobial activity of W Sextracts

Antibacterial Activity 1000 µg / disc (mm) Extracts and standards	Gram Positive Bacteria		Gram Negative Bacteria		Antifungal Activity Yeast
	<i>S.a</i>	<i>E.f</i>	<i>E.c</i>	<i>Pa</i>	<i>C.a</i>
Amoxicillin	38	34	26	—	NT
Fluconazole	NT	NT	NT	NT	15
acetone insoluble fraction of leaves	28	24	—	—	—
Methanol extract of leaves	24	14	—	—	—
Chloroform extracts of leaves	22	16	—	—	—
Ethyl acetate extract of leaves	19	14	—	8	—
Butanol extracts of leaves	16	—	—	—	—
Petroleum ether extracts of leaves	—	—	—	—	—
Mother liquorextract of leaves	—	—	—	—	—
acetone insoluble fraction of stems	25	23	—	—	—
Methanol extracts of stems	12	—	—	—	—
Chloroformextract of stems	17	12	—	—	—
Ethyl acetateextract of stems	11	8	—	—	—
Butanolextract of stems	13	—	—	—	—
Petroleum etherextract of stems	—	—	—	—	—
Mother liquorextract of stems	—	—	—	—	—
Methanol extracts of seeds	—	—	—	—	—
Chloroformextracts of seeds	—	—	—	—	—
Ethyl acetate extracts of seeds	—	—	—	—	—
Butanol extracts of seeds	—	—	—	—	—
Petroleum ether extracts of seeds	—	—	—	—	—
Mother liquorextract of seeds	—	—	—	—	—

S.a: *Staphylococcus aureus*; *E.f*: *Enterococcus Faecalis*; *E.c*: *Escherichia Coli*; *Pa*: *Pseudomonas Aerugenosa* ; *C.a*: *Candia albican*; NT: Not Tested; —: No Zone of inhibition

that the methanol extract had the highest TPC. The obtained results of TFC indicated that, the butanol extract of stems (BE-2) exhibited the highest TFC with 66.2 mg of QE/g powder weight which is the major contributor to scavenge the free radicals in oxidation pathways. Moreover, ethyl acetate extract of leaves (EE-1) showed a comparable TFC with 65.6 mg of QE/g powder weight when it was compared with BE-2. However, the methanol extract of leaves (ME-1) exhibited the lowest TFC²⁴.

In Vitro Antimicrobial Activity of WS

About 20 extracts of different parts of WS were tested for their bioactivity against bacterial and fungal strains at concentration (1000) µg/disc. From the results which recorded in table (5) it was observed that, the gram positive bacteria are sensitive towards some extracts, but the gram negative bacteria showed resistance against the extracts. The fungus *Candida albicans* did not show any inhibition signs for all extracts in this concentration. The acetone insoluble fraction of leaves and stems showed high activity against gram positive bacteria. But the gram negative bacteria showed resistance. The methanol extracts of leaves showed inhibition zone against *S. aureus* and *E. faecalis*. Methanol extract of stems had some activity against *S. aureus* only. Methanol extract of seeds did not exhibited any activity against any type from the tested microbes. Chloroform extracts of leaves and stems showed marked activity against *S. aureus* and *E. faecalis*. However, chloroform extract of seeds did not showed any activity against any type from the tested microbes. These results are disagree with that reported by Singariya et al., in 2012²⁵ were they found that, the chloroform extract of WS fruits showed low activity against gram negative bacteria (*P. aeruginosa*). Moreover, ethyl acetate extract of leaves showed a weak activity against gram negative bacteria *P. aeruginosa* (inhibition zone, 8 mm) in addition to its moderate activity against gram positive bacteria *S. aureus* and *E. faecalis*. Our results were disagree to the results of Sundaram et al., in 2011²⁶ where, they noticed that, the ethyl acetate extract of leaves possesses a great inhibition against gram positive bacteria and gram negative bacteria especially *P. aeruginosa*. Ethyl acetate extract of stems showed activity against *S. aureus* and *E. faecalis* while, ethyl acetate extract of seeds showed negative

results for all tested microbes. The butanol extract of leaves showed some activity against *S. aureus*. Butanol extract of stems showed some activity against *S. aureus* only. Butanol extract of seeds did not show any activity against any type of tested microbes. Petroleum ether and mother liquor extracts of all subjected parts gave negative results for all tested microbes. All extracts showed positive results with inhibition zones less than that of the standard drugs Amoxicillin except ethyl acetate extract of leaves against *P. aeruginosa*.

CONCLUSION

From the present study, it is possible to conclude that, 9-Octadecenamide is a major compound in volatile oils of leave and stems of WS, in addition the plant is consider as rich source of saturated and unsaturated fatty acids. As well as reasonable quantities from flavonoids and phenols. Furthermore, several compounds which were identified by GC/MS may be responsible for the activity of this plant as antimicrobial agent against gram positive bacteria specially leaves and stems extracts. These findings of our research could be beneficial in the future to complete a further study of biological activity and other possible therapeutic uses of the plant.

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