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IDENTIFICATION OF CHEMICAL COMPOUNDS, ANTIMICROBIAL AND ANTIOXIDANT EFFECTS OF EXTRACTS FROM SEEDS, STEMS AND ROOTS OF SYMBOPOGON SCHOENANTHUS

Ali.A. Eltayeib and Nahla Agab

Department of Chemistry, Faculty of Science, Kordofan University, Sudan.

ABSTRACT

The aim of this study was to identify chemical composition, antimicrobial and antioxidant activity of extracts of stems and roots (mixture 1:1) and seeds from mahareb plant. The seeds, stems and roots were collected from alshareef allged (north kordofan state), during March (2018), dried under shade, grinded and then extracted by maceration method using 99% methanol, petroleum ether and distilled water. Photochemical screening revealed the presence of flavonoids, terpenoids, saponins and tannins with high concentration except alkaloids with low concentration in extracts. Crude extracts and tetracycline with different concentrations (40, 60, 70 and 90 mg/ml) were applied against four bacterial strains (two Gram positive: Sterbeto cocai (S.c), Staphylococcus aureus (ATCC 25923); and two Gram negative: Escherichia Coli (E.c)(ATCC 25922), Pseudomonas aerruginosa (P.s.a) (ATCC 27853) and with 100 mg/ml against one type of fungus (candida albicans (ATCC 7596) using agar plate well-diffusion method. According to this method the inhibition zone less than 14 resistance, 14 to 18 medium and larger than 18 mm sensitive. Methanol seeds extract at 90mg/ml showed activity against P.s.a (22mm) and E.c (17mm). Methanol roots and stems extracts at 70mg/ml showed activity against P.s.a (17mm). Tetracycline at 90 and 60mg/ml showed activity against (E.c 20mm, S.c 16mm) and (E.c 16mm) respectively. The extracts showed no activity against candida albicans. The free radical scavenging activity of the crude extracts was evaluated using 2,2 -Di (4-tert-octylphenyl)-1-picryl hydrazyl stable free radical (DPPH). The extracts showed a DPPH scavenging activity of 16±0.08, 85±0.00, 46±0.08 (for seeds extracted by water, methanol and petroleum ether respectively) and 10±0.07, 56±0.08, 4±0.07 (for stems and roots mixture extracted by water, methanol and petroleum ether respectively). The standard propyl gallate showed 95±0.02 scavenging activity. Analysis of the crude extracts by GC-MS showed 56, 52, 5, 9, 54, 38 compounds for seeds, stems and roots extracted by methanol, water and

petroleum ether respectively.

Keywords: Symbopogon schoenanthus, stems, roots, chemical compounds.

1. INTRODUCTION

Plants, which have one or more of its parts having substance that can be used for treatment of diseases, are called medicinal plants (Sofowora, 1982). Medicines derived from plants are widely famous due to their safety, easy availability and low cost (Iwu et al, 1999). Herbal medicines may include whole parts of plant or mostly prepared from leaves, roots, bark, seeds and flowers of plants. They are administered orally, inhaled or directly applied in the skin (Westh et al., 2004). Medicinal herbs are more significant to the health of individual and community. The medicinal value of these plants lies in bioactive phytochemical constituent that produce definite physiological action on the human body (Hill, 1952). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more (Solecki 1975). There is up to 80 percent of people still on herbal remedies for their health care (Farnsworth et al., 1985). Sudan is a rich country with indigenous herbal resources. This is due to the variation in climate, rainfall and soils.

It is estimated that Sudan encompasses more than 3156 species belonging to 1137 genera and 170 families (Broun and massay, 1929, Elamin, 1990). Therefore, there are large numbers of medicinal plants in Sudan used traditionally against different diseases. In spite of recent domination of synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous (Raskin et al., 2002). Compared with chemical synthesis, plant derived natural products represent an attractive source of biologically active agents since they are natural and available species (Ghosh et al., 2008). Plants synthesize many compounds called primary metabolites that are critical to existence. Development countries are still using medicinal plant for their health care (Kim, 2005). Nowadays, plants countries to be the major source of medicine in rural region of developing countries (Chitme et al., 2003).

Traditional medicine has wide acceptability and along history. Indeed, majority of the people use these medications at one time or another and this presupposes the efficacy and safety of plant materials used in ethno medicines. It could not be ascertained when and how the practitioners first introduced remedy or prescription (Igoli et al., 2003). Ethno medical studies are today, recognized as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents. Traditional medicine is concerned with the study of medical systems from the native's point of view. Native categories and explanatory models of illness, including an etiology, symptoms, courses of sickness, and treatments are investigated. Most of the Sudanese people in rural areas rely on traditional medicine for the treatment of many infectious diseases (Elkamli and Elkhalifa, 1997). Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in the industrialized countries. In china for example, traditional herbal preparations account for 30% - 50% of

total medicinal consumption. In Ghana, Mali, Nigeria, and Zambia, the fires line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicine at home (Bannerman et al., 1993). Ethno medical studies are today, recognized as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents. Early studies on indigenous medical systems were mostly limited in focus on witchcraft and illness caused by super-natural forces, and on specialists such as folk healers, and shamans (Fabrega and Silver, 1973).

MATERIALS AND METHODS

Plant material:

The roots, seeds and stems of *cymbopogon schonenanthus rufescens* were collected in April 2018, from plant field in Alshreef Aljad about 10 Km from Oum Kradium (North Kordofan State). The plant was authenticated by Herbarium National Center for Medicinal Research and Aromatic plants Institute. The plant materials were shade-dried, cleaned and grinded by a mechanical grinder. Equal amounts of stems and roots were mixed as one sample. The grounded samples were stored at room temperature to be ready for further extraction.

Methods:

Preparation of crude extracts:

Hundred grams of each of the dried powder (seeds and the mixture of stems and roots) were macerated exhaustively for three days at room temperature with1000 ml of methanol and petroleum ether and for four hours with 1000 ml of water. The extracts were filtered and the obtained products were weighed prior to further analysis.

Phytochemical screening of the crude extracts:

The dried extracts were reconstituted in methanol, pettroleum ether and water and then subjected to qualitative tests for the presence of phytochemical compounds in the different extracts according to methods described by Harborne (1998).

Blood agar base

The blood agar base is prepared by dissolving 40g of media in 1 liter
distilled water, boiled to dissolve completely, sterilized at 121 º C and 15-165 atmospheric pressure for 15 minutes, cooled to 50 º C, aseptically 10% fresh blood was added, mix with gentle rotation and poured in the petri dish plates. The four types of isolated bacteria are cultured by using loop and benzene lamp, incubated for 24 hours at 37 º C. Holes of known diameter (8mm) were made for each of the surface of the grown colonies of bacteria. In each plate four holes were made with another one at the center as control filled with distilled water. Alternate holes are filled with the extracts (90, 70, 60, 40mg/ml) and allowed to diffuse at room temperature for two hours then incubated at 37 º C for 18 hours. It is noticed that the color of the extract of each holes is spread. Using verna the diameter of inhibition zone was calculated as follows: inhibition zone diameter (mm) = the diameter of extract – the diameter of the hole (8mm) (Forbes et al., 1990).

Preparation of fungus suspension

The fungal cultures were maintained on sabouraud dextrose agar, incubated at 25 º C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

Bacterial microorganisms

Sterbeto Cocai NCTC8235 (Gram +ve bacteria)
Staphylococcus aureus ATCC25923 (Gram +ve bacteria)
Escherichia Coli ATCC25922 (Gram -ve bacteria)
Pseudomonas aeruginosa ATCC27853 (Gram -ve bacteria)
National Collection of Type Culture (NTCC), Colindale, England.
American Type Collection (ATCC) Rockville, Maryland,USA.

Fungus microorganism

Candida albicans ATCC7596

Gas chromatography-mass spectrometry (GC-MS) conditions:

The qualitative analysis of the sample was carried out by using GC-MS technique model (GC-MS-QP2010-Ultra) from Japanese Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.69 ml/min, the temperature program was started from 50oC with rate 7oC /min to 180oC then the rate was changed to 10oC/min reaching 300oC as final temperature degree with 2 minutes as hold time , the injection port temperature was 300oC , the ion source temperature was 200oC and the interface temperature was 250oC. The sample was analyzed by using scan mode in the range of 40-600m/z mass to charge ratio and the total run time was 28 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST).
DPPH radical scavenging assay:

The DPPH radical scavenging was determined according to the method of Shimada et al., (1992) with some modifications. In 96-wells plate, 0.5mg from each sample was allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl hydrazyl stable free radical (DPPH) for half an hour at 37oC. The concentration of DPPH was kept as 300µM.The test samples were dissolved in dimethyl sulfoxide (DMSO) while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multi-plate reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated as control group. All tests and analysis were run in triplicate.

RESULTS AND DISCUSSION

Depending on their polarity solvents will extract varying quantities of components from the plant material,

for this reason solvents of different polarities were used.

Some of the physical properties and yields of *cymbopogon schoenanthus* extracts were shown in table 1. All the solvents produced the same yield except the seeds with petroleum ether produced higher yield. An increase in the extracted material in seeds by petroleum ether could be related to high content of non polar compounds in this part of the plant.

Table 1: Physical properties of *cymbopogon schoenanthus* extracts

Solvent	Color	Consistency	Yield (gm)
P.ether(seed)	Greenish	Liquid	2.6
P.ether (roots, stems)	Greenish	Liquid	1.7
Water(seed)	Brownish	Solid	1.6
Water(roots, stems)	Brownish	Solid	1.6
Methanol(seed)	Yellowish	Liquid	1.6
Methanol(roots,stems)	Yellowish	Liquid	1.6

P. ether: petroleum ether

Phytochemical screening

The results of the phytochemical screening of the extracts were shown in tables (2) and (3). Results showed high concentration of terpenoids for both seeds and the mixture of stems and roots (Methanol, petroleum ether and water extracts) but low concentrations of flavonoids and saponins for seeds and stems, roots mixture. Tannins and alkaloids not detected in the methanol and water extracts.

Table 2: Phytochemical screening of the seed's extracts

Component	Methanol	Petroleum ether	Water extract
Alkaloids	-	-	-
Saponins	+	+	+++
Flavonoids	+	-	-
Tannins	-	-	-
Terpenoids	+++	+++	+++

+++: High concentration, +: Low concentration, -: Not detected

Table 3: Phytochemical screening of the stems and roots mixture

Component	Methanol	P.ether	Water extract
Alkaloids	-	-	-
Saponins	+	+	+
Flavonoids	+++	+	+
Tannins	+++	-	-
Terpenoids	+++	+++	+++

Assessment of antimicrobial activity of the extracts and tetracycline:

Assessment of antibacterial activity of *cymbopogon schoenanthus* extracts and tetracycline with different concentrations (40, 60, 70, 90 mg/ml) were carried out against four types of bacteria, two gram positive (Sterbeto cocai and staphylococcus) and two gram negative (Escherichia coli and Pseudomonas).The extracts also examined against one fungus (*Candida albicans*) with 100 mg/ml. The assessment of antimicrobial activity of the extracts and tetracycline were shown in tables (4),(5) and (6).

The extract of seeds extracted by methanol at 90mg/ml exhibited activity against pseudomonas aeruginosa (22mm) and Escherichia Coli (17mm). The methanol extract of roots and stems at 70mg/ml showed activity against pseudomonas aeruginosa (17mm). Other concentrations of the extracts showed no activity against the tested bacteria also the extracts showed no activity against the tested fungus (Candida albicans). Tetracycline at 90mg/ml exhibited activity against Escherichia Coli (20mm) and Sterbeto cocai (16mm), at 60mg/ml showed activity against Escherichia Coli (16mm). Other concentrations of tetracycline showed no activity towards the tested bacteria.

Table 4: Antibacterial activity of extracts against four types of bacteria (inhibition zone in mm)

Plant material	Solvent	Concentration mg/ml	P.s.a	E.c	S.a	S.c
Seeds	P. ether	90	9	7	7	10
~	Methanol	90	22	17	12	9
~	Water	90	13	9	8	13
Roots,stems	P. ether	90	10	9	7	10
~	Methanol	90	7	7	5	8
~	Water	90	9	8	9	7
Seeds	P. ether	70	5	6	3	5
~	Methanol	70	9	7	8	8
~	Water	70	12	11	10	6
Roots,stems	P. ether	70	8	10	9	11
~	Methanol	70	17	12	13	11
~	Water	70	7	9	8	8
Seeds	P. ether	60	3	5	6	6
~	Methanol	60	10	8	9	11
~	Water	60	9	6	6	5
Roots,stems	P. ether	60	4	2	3	5
~	Methanol	60	5	3	6	4
~	Water	60	4	7	8	8
Seeds	P. ether	40	2	3	5	3
~	Methanol	40	6	4	5	6
~	Water	40	8	6	5	3

E.c = *Escherichia coli*, P.s.a = *Pseudomonas aeruginosa*
S.a = *Staphylococcus aureus*, S.c = *Sterbeto cocai*, C.a= *Candida albicans*

According to the method used for antimicrobial assessment the inhibition zone less than 14 resistance, 14 to 18 medium and larger than 18 mm sensitive.

Table 5: Antibacterial activity of tetracycline against four types of bacteria (inhibitition zone in mm)

Concentration mg/ml	P.s.a	E.c	S.a	S.c
90	14	20	9	16
70	10	14	7	11
60	12	16	8	13
40	11	13	6	12

Table 6: Antifungal activity of extracts against candida albican (inhibitition zone in mm)

Plant material	Solvent	Concentration mg/ml	C.a
Seeds	P.ether	100	-
~	Methanol	100	-
~	Water	100	-
Roots,stems	P.ether	100	-
~	Methanol	100	-
~	Water	100	-

Chemicals compounds of different extracts

The chemical compounds identified in the different crude extracts from the stems, roots and seeds were shown in figures from (4) to (9) and tables (7) to (12) below. The chemical compounds with their retention time, peak area and area % were shown in the tables.

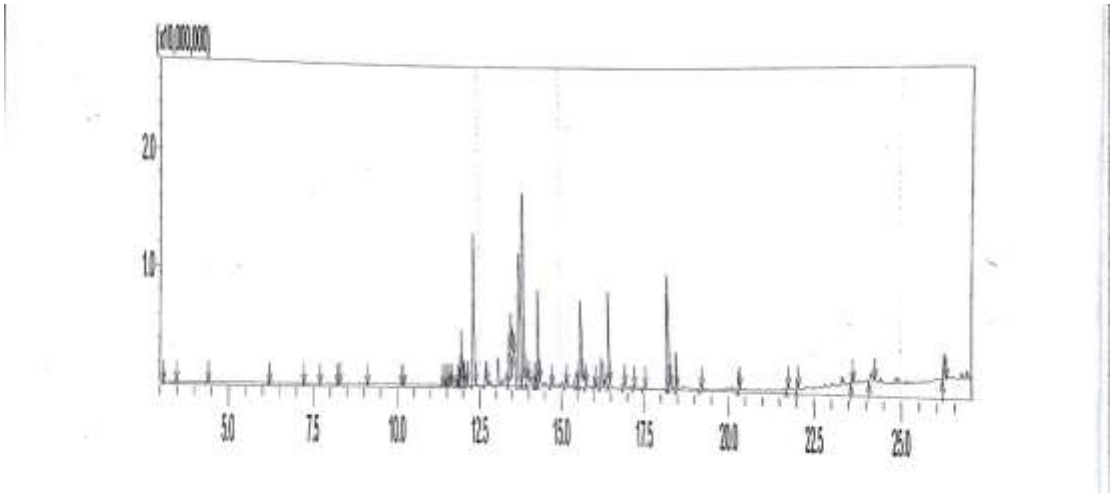

Figure 4: Gas chromatogram of the chemical compounds of seeds extracted by petroleum ether

Table 7: Chemical compounds of petroleum ether extract from the seeds

Peak Report TIC

Peaks	R.Time	Area	Area%	Name
1	3.081	101213	0.03	Pentanoic acid, 4-methyl-, methyl ester
2	3.446	181232	0.05	Hexanoic acid, methyl ester
3	4.361	369255	0.10	Heptanoic acid, methyl ester
4	6.157	570986	0.16	Octanoic acid, methyl ester
5	7.184	316765	0.09	Methyl 6-methyloctanoate
6	7.631	165532	0.05	Nonanoic acid, methyl ester
7	8.143	406401	0.11	Geraniol
8	8.239	356101	0.10	2-Cyclohexen-1-one, 3-methyl-6-(1-methy
9	9.074	145894	0.04	Decanoic acid, methyl ester
10	10.070	171583	0.05	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(
11	10.169	1333585	0.37	.alpha.-Bulnesene
12	11.343	425412	0.12	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7
13	11.452	530997	0.15	.beta.-ylangene
14	11.474	909544	0.25	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,
15	11.539	523007	0.14	Alloaromadendrene
16	11.618	4255386	1.18	.alpha.-acorenol
17	11.790	1645544	0.45	Benzene, 1-methyl-4-(1,2,2-trimethylcycl
18	11.862	1874025	0.52	.alfa.-Copaene
19	11.902	1212814	0.34	Humulane-1,6-dien-3-ol
20	11.952	8304643	2.29	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-c
21	12.026	3834516	1.06	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9
22	12.095	1799249	0.50	Longifolene
23	12.339	36986044	10.22	Cyclohexanemethanol, 4-ethenyl-.alpha.,.
24	12.697	4725891	1.31	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-c
25	13.410	10823288	2.99	8-epi-.gama.-eudesmol
26	13.468	6138674	1.70	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahy
27	13.525	5686033	1.57	.alpha.-Cadinol
28	13.730	55153965	15.24	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-
29	13.857	65380439	18.07	1-Naphthalenol, decahydro-1,4a-dimethyl
30	13.971	2491931	0.69	1,4-Methanoazulen-9-ol, decahydro-1,5,5,
31	14.175	1232500	0.34	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),
32	14.233	1249044	0.35	Methyl tetradecanoate
33	14.300	17142810	4.74	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-
34	14.691	2998676	0.83	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane
35	15.141	2433976	0.67	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-c
36	15.458	2819305	0.78	Caryophyllene oxide
37	15.613	25387828	7.02	2-Methyl-5-(2,6,6-trimethyl-cyclohex-1-e
38	15.732	2205039	0.61	7R,8R-8-Hydroxy-4-isopropylidene-7-me
39	16.013	2579016	0.71	Cubenol
40	16.226	5992431	1.66	Methyl dihydroisosteviol
41	16.452	15725255	4.35	Hexadecanoic acid, methyl ester
42	16.879	917222	0.25	n-Hexadecanoic acid
43	17.198	535151	0.15	Hexadecanoic acid, 14-methyl-, methyl es
44	17.479	442926	0.12	Hexadecanoic acid, 15-methyl-, methyl es
45	18.207	18741554	5.18	9,12-Octadecadienoic acid (Z,Z)-, methyl
46	18.254	22519934	6.22	9-Octadecenoic acid (Z)-, methyl ester
47	18.468	5482152	1.51	Methyl stearate
48	19.189	1181393	0.33	trans-Geranylgeraniol
49	20.317	2934866	0.81	Eicosanoic acid, methyl ester
50	21.740	567460	0.16	1-Decanol, 2-hexyl-
51	22.020	1415310	0.39	Docosanoic acid, methyl ester
52	23.599	1535433	0.42	Tetracosanoic acid, methyl ester
53	24.151	3099125	0.86	.gamma.-Sitosterol
54	26.212	5919871	1.64	Octadecanal

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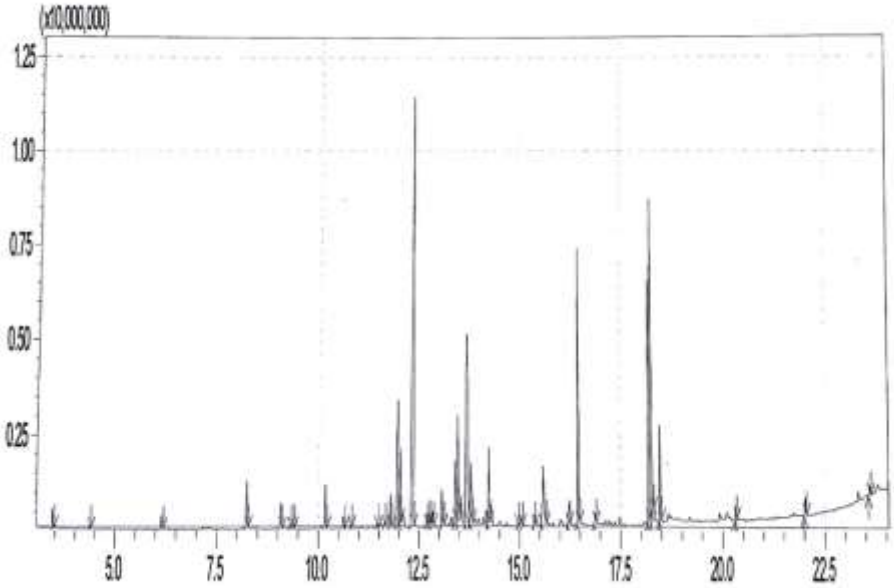

Figure 5: Gas chromatogram of the chemical compounds of the stems and roots extracted by petroleum ether

Table 8: Chemical compounds of petroleum ether extract from the stems and roots

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.438	711840	0.52	Hexanoic acid, methyl ester
2	4.355	138746	0.10	Pentanoic acid, 4-methyl-, methyl ester
3	6.150	636865	0.47	Octanoic acid, methyl ester
4	8.232	2344205	1.71	2-Cyclohexen-1-one, 3-methyl-6-(1-methyl
5	9.069	1164348	0.85	Decanoic acid, methyl ester
6	9.304	234048	0.17	2-Cyclohexen-1-one, 2-methyl-5-(1-methyl
7	9.386	119870	0.09	1,3,6-Heptatriene, 2,5,5-trimethyl-
8	10.169	1939290	1.42	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1
9	10.623	509841	0.37	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimeth
10	10.804	156176	0.11	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,
11	11.445	256883	0.19	.beta.-ylangene
12	11.643	274196	0.20	.alpha.-Guaiene
13	11.783	1683386	1.23	Undecanoic acid, 10-methyl-, methyl ester
14	11.952	6121708	4.48	6-epi-shyobunol
15	12.023	3138110	2.29	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9
16	12.330	27547052	20.14	Cyclohexanemethanol, 4-ethenyl-.alpha.,.
17	12.694	602279	0.44	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cy
18	12.747	464986	0.34	1H-Cycloprop[e]azulen-7-ol, decahydro-1,
19	12.829	707324	0.52	Caryophyllene oxide
20	13.063	1879017	1.37	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-
21	13.396	2932256	2.14	.gama.-eudesmol
22	13.452	4500280	3.29	Agarospirol
23	13.672	15469346	11.31	2-Naphthalenemethanol, decahydro-.alpha
24	13.790	3333420	2.44	1-Naphthalenol, decahydro-1,4a-dimethyl-
25	14.227	3232952	2.36	Methyl tetradecanoate
26	14.949	248048	0.18	Tridecanoic acid, methyl ester
27	15.045	338529	0.25	Tridecanoic acid, 12-methyl-, methyl ester
28	15.363	498330	0.36	Pentadecanoic acid, methyl ester
29	15.583	4376677	3.20	(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-m
30	16.221	1150139	0.84	(-)-Spathulenol
31	16.452	13837040	10.12	Hexadecanoic acid, methyl ester
32	16.871	469022	0.34	n-Hexadecanoic acid
33	18.204	13138805	9.61	9,12-Octadecadienoic acid (Z,Z)-, methyl e
34	18.254	16232084	11.87	9-Octadecenoic acid (Z)-, methyl ester
35	18.467	4350436	3.18	Methyl stearate
36	20.316	680930	0.50	Eicosanoic acid, methyl ester
37	22.020	868819	0.64	Docosanoic acid, methyl ester
38	23.598	471679	0.34	Tetracosanoic acid, methyl ester
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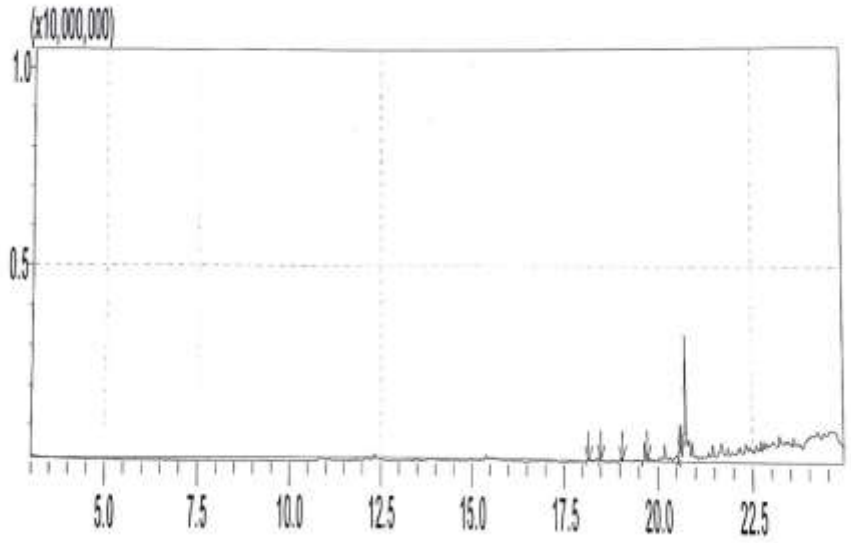

Figure 6: Gas chromatogram of the chemical compounds of the seeds extracted by water

Table 9: Chemical compounds of the seeds extracted by water

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	18.134	276105	8.00	3,9-Dimethyltricyclo[4.2.1.1(2,5)]decan-9-(
2	18.467	305744	8.86	1H-Cycloprop[e]azulen-4-ol, decahydro-1,
3	19.025	263051	7.62	9-Undecenal, 2,10-dimethyl-
4	19.659	1017892	29.50	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-
5	20.595	1587863	46.02	Globulol
		3450655	100.00	

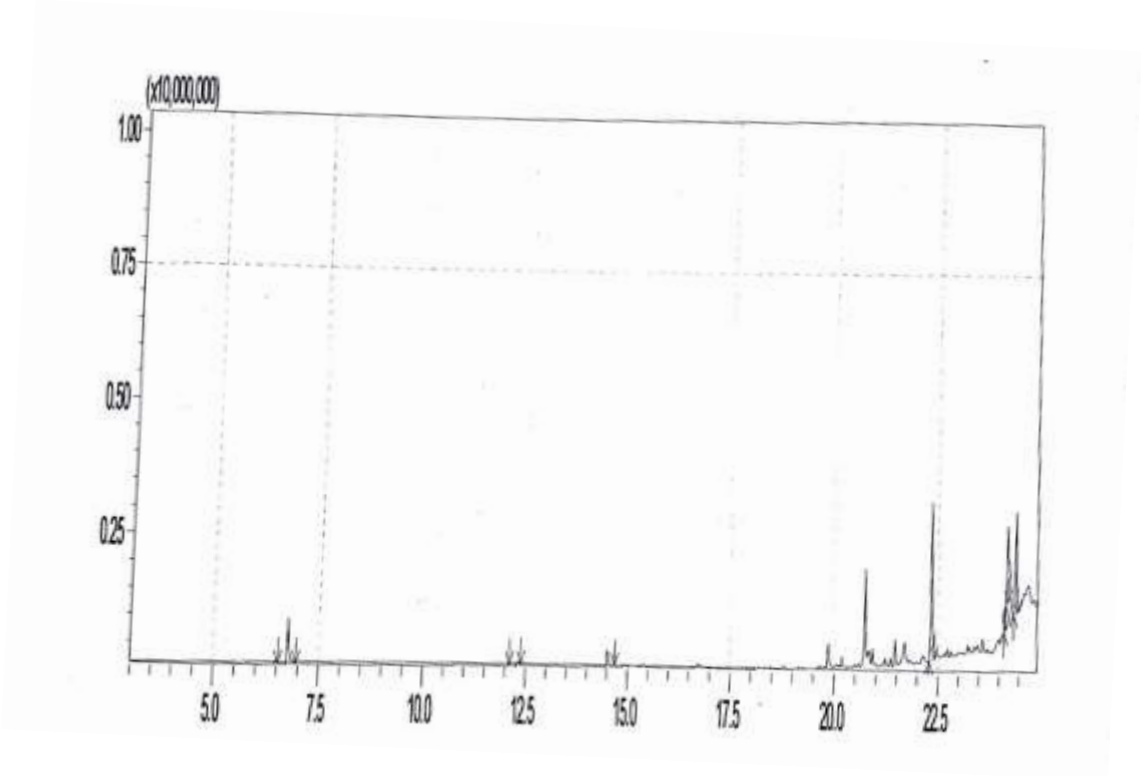

Figure 7: Gas chromatogram of the chemical compounds of the stems and roots extracted by water

Table 10: Chemical compounds of the stems and roots extracted by water

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	6.476	203037	1.01	1-Hexanol, 2-ethyl-
2	6.758	2702245	13.47	Pantolactone
3	12.086	55223	0.28	2-Oxa-spiro[4.5]dec-8-ene-1,7-dione, 4,6-d
4	12.328	179600	0.90	2-Methoxy-4-vinylphenol
5	14.506	1118492	5.58	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-
6	22.345	7526639	37.52	n-Hexadecanoic acid
7	24.150	864195	4.31	9,12-Octadecadienoic acid (Z,Z)-
8	24.188	3226706	16.09	9-Octadecenoic acid, (E)-
9	24.397	4183378	20.85	Octadecanoic acid
		20059515	100.00	

Figraphy.

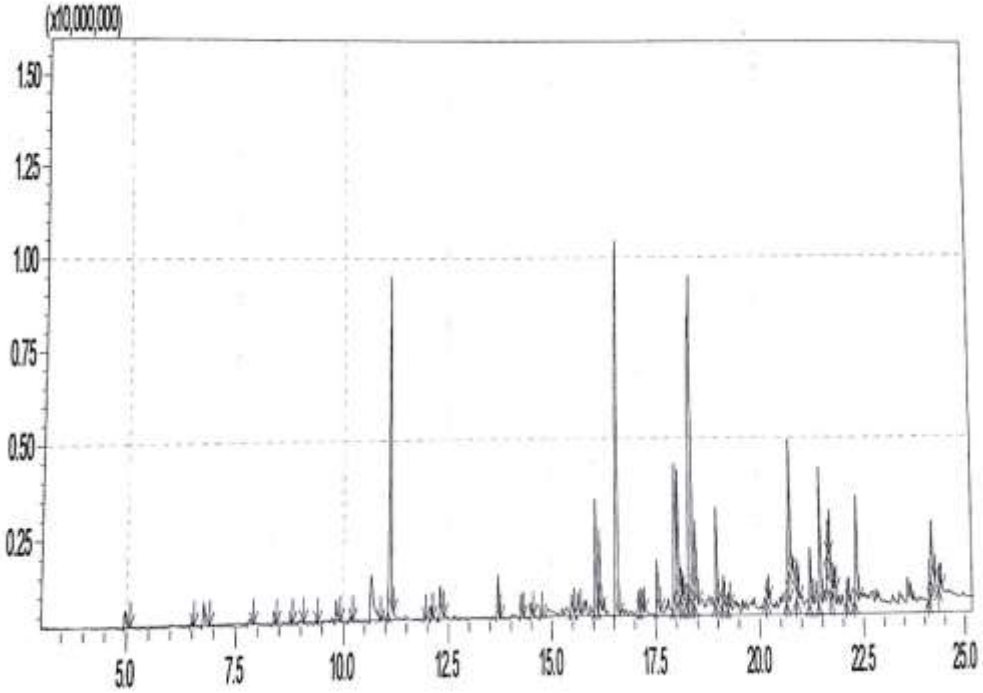

Figure 8: Gas chromatogram of the chemical compounds of the stems and roots extracted by methanol

Table 11: Chemical compounds of the stems and roots extracted by methanol

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	4.960	1474309	0.64	2,5-Furandione, 3-methyl-
2	6.493	317080	0.14	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-e
3	6.761	1924801	0.83	Pantolactone
4	7.808	758727	0.33	Phenol, 2-methoxy-
5	8.392	652238	0.28	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylet
6	8.764	439202	0.19	Eucalyptol
7	8.971	420157	0.18	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox
8	9.345	215330	0.09	2-(2-Benzyloxy-4-methyl-cyclohex-3-enyl)-
9	9.826	1319906	0.57	.alpha.-Terpineol
10	10.159	391952	0.17	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylet
11	10.647	7269041	3.15	Benzofuran, 2,3-dihydro-
12	11.115	24832768	10.77	2-Cyclohexen-1-one, 3-methyl-6-(1-methyl
13	11.932	436858	0.19	Thymol
14	12.088	787156	0.34	2-Oxabicyclo[2.2.2]octan-6-one, 1,3,3-trim
15	12.306	2434638	1.06	2-Methoxy-4-vinylphenol
16	13.698	2468652	1.07	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1
17	14.256	1396987	0.61	Caryophyllene
18	14.496	1049309	0.45	Cedrene
19	14.730	297944	0.13	Caryophyllene oxide
20	15.476	1412147	0.61	Bicyclo[5.3.0]decane, 2-methylene-5-(1-me
21	15.626	1474321	0.64	.alpha.-Guaiene
22	16.064	7146471	3.10	6-epi-shyobunol
23	16.154	4464856	1.94	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-
24	16.584	28356170	12.29	Cyclohexanemethanol, 4-ethenyl-.alpha.,.a
25	17.100	1344899	0.58	1H-Cycloprop[e]azulen-7-ol, decahydro-1,
26	17.188	1433512	0.62	11,11-Dimethyl-spiro[2,9]dodeca-3,7-dien
27	17.546	3668917	1.59	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-
28	17.987	13418089	5.82	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-o
29	18.055	7353677	3.19	Agarospirol
30	18.118	1393903	0.60	Hinesol
31	18.183	2253202	0.98	Guaiol
32	18.341	23376235	10.13	2-Naphthalenemethanol, decahydro-.alpha
33	18.382	17917800	7.77	.gama.-eudesmol
34	18.480	5727971	2.48	1-Naphthalenol, decahydro-1,4a-dimethyl-
35	18.977	5524655	2.40	Illudol
36	19.136	1697748	0.74	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien
37	19.264	853041	0.37	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-
38	20.189	938645	0.41	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-o
39	20.701	5205748	2.26	1-Heptatriacotanol
40	20.746	7701983	3.34	Ledol
41	20.917	2214222	0.96	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8
42	21.222	3246021	1.41	Longifolenaldehyde
43	21.466	8392241	3.64	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydr
44	21.662	1378896	0.60	Aristolene epoxide
45	21.694	4392345	1.90	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol
46	21.800	1172312	0.51	Dihydro-isosteviol methyl ester
47	22.120	1385154	0.60	Gitoxigenin
48	22.346	7476914	3.24	n-Hexadecanoic acid
49	24.139	2802263	1.21	9,12-Octadecadienoic acid (Z,Z)-
50	24.185	4116346	1.78	9-Octadecenoic acid, (E)-
51	24.379	1266889	0.55	Octadecanoic acid
52	26.756	1268057	0.55	4-tert-Butoxystyrene
		230662705	100.00	

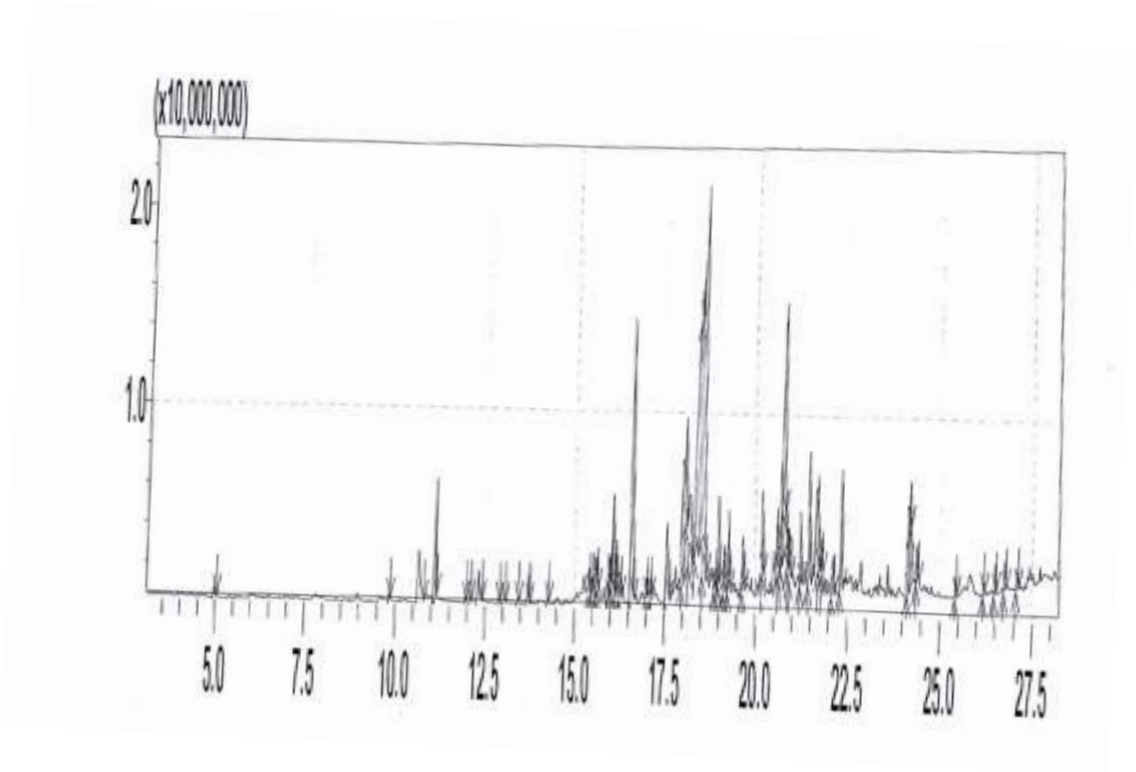

Figure 9: Gas chromatogram of the chemical compounds of the seeds extracted by methanol

Table 12: Chemical compounds of the seeds extracted by methanol

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	4.957	1407136	0.25	2,5-Furandione, 3-methyl-
2	9.829	618649	0.11	.alpha.-Terpineol
3	10.631	13514426	2.45	Benzofuran, 2,3-dihydro-
4	11.103	13058633	2.36	2-Cyclohexen-1-one, 3-methyl-6-(1-methy
5	11.935	518189	0.09	Thymol
6	12.094	538349	0.10	2-Oxabicyclo[2.2.2]octan-6-one, 1,3,3-trin
7	12.307	5061374	0.92	2-Methoxy-4-vinylphenol
8	12.882	528834	0.10	.alpha.-Cubebene
9	13.025	1014879	0.18	Phenol, 2,6-dimethoxy-
10	13.413	678038	0.12	Copaene
11	13.701	3654248	0.66	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(
12	14.259	946335	0.17	Caryophyllene
13	15.409	3270473	0.59	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,
14	15.481	1836379	0.33	Bicyclo[5.3.0]decane, 2-methylene-5-(1-m
15	15.583	4402335	0.80	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1
16	15.625	2996927	0.54	2H-3,9a-Methano-1-benzoxepin, octahyd
17	15.945	5077227	0.92	.gamma.-Muurolene
18	15.997	2416669	0.44	Selina-6-en-4-ol
19	16.075	13147483	2.38	Cubenol
20	16.161	5872438	1.06	6-epi-shyobunol
21	16.261	3184567	0.58	cubedol
22	16.611	49345301	8.93	Cyclohexanemethanol, 4-ethenyl-.alpha.,.
23	17.004	2315192	0.42	1H-Cycloprop[e]azulen-4-ol, decahydro-1
24	17.109	1765421	0.32	1H-Cycloprop[e]azulen-7-ol, decahydro-1
25	17.564	13553054	2.45	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a
26	18.016	27422342	4.96	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-(
27	18.088	15070823	2.73	Agarospirol
28	18.200	19938843	3.61	.alpha.-Cadinol
29	18.455	61198049	11.08	2-Naphthalenemethanol, decahydro-.alph
30	18.482	2401001	0.43	1-Naphthalenemethanol, 1,2,3,5,6,7,8,8a-(
31	18.594	83575592	15.13	1-Naphthalenol, decahydro-1,4a-dimethyl
32	18.900	2127836	0.39	(-)-Spathulenol
33	18.999	8401661	1.52	Illudol
34	19.150	5185306	0.94	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trie
35	19.273	8288572	1.50	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-
36	19.669	5778012	1.05	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane
37	20.202	7929878	1.44	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-(
38	20.609	6873815	1.24	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-
39	20.723	13902197	2.52	(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-(
40	20.806	36516262	6.61	Ledol
41	20.862	4600410	0.83	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,
42	20.934	4995395	0.90	7R,8R-8-Hydroxy-4-isopropylidene-7-me
43	21.239	7199013	1.30	Longifolenaldehyde
44	21.484	15748736	2.85	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahyd
45	21.712	8638209	1.56	5-Azulenemethanol, 1,2,3,3a,4,5,6,7-octah
46	21.814	3005175	0.54	4-Isopropenyl-4,7-dimethyl-1-oxaspiro[2.
47	22.125	5125840	0.93	Gitoxigenin
48	22.356	16917034	3.06	n-Hexadecanoic acid
49	24.155	13326687	2.41	9,12-Octadecadienoic acid (Z,Z)-
50	24.201	3245216	0.59	9-Octadecenoic acid, (E)-
51	24.384	3640617	0.66	Octadecanoic acid
52	25.425	1702073	0.31	Acetic acid, [(Z,Z)-3,7,11-trimethyl-2,6,10
53	26.176	1115801	0.20	Eicosanoic acid
54	26.491	2462282	0.45	Propanoic acid, 2,2-dimethyl-, [(E,E)-3,7,

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Peak#	R.Time	Area	Area%	Name
55	26.754	3689247	0.67	4-tert-Butoxystyrene
56	27.080	1760157	0.32	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-,
		552504637	100.00	

Antioxidant activity

The results of antioxidant for the extracts of seeds, roots and stems were shown in table 13. The seed extracts by methanol showed the highest antioxidant activity as compared to the rest of extracts. The water extracts showed the lowest antioxidant activity as compared to other solvents extracts.

Table 13: Radical scavenging activity of the different crude extracts the seeds, stems and roots samples by DPPH method

Plant material	Solvent	%of activity ±SD
Seeds	Water	16± 0.08
	Methanol	85± 0.00
	Petroleum ether	46± 0.08
Stem and roots	Water	10±0.07
	Methanol	56±0.08
	Petroleum ether	4±0.07
Standard	Propyl Gallate	95±0.02

SD: standard deviation

$$\text{Percentage of activity} = 100 - \frac{\textbf{absorption of the sample}}{\textbf{absorption of the blank}} \times 100$$

CONCLUSION

The results do not show which compound is responsible for the antimicrobial and antioxidant activity; hence, the whole plant could be of use as a source of antioxidant and antimicrobial. Mainly methanol extracts can be use as antioxidant and antimicrobial.

REFERENCES

Bannerman, R. H.; Burton, J. and Cheien, W. (1993). Traditional medicine and health care converge. World Health Organization, Genera, Switzerland.

Broun, A. F. and Massay, R. E. (1929). Flora of the Sudan.Wellington house, London.

Chitme, H.; Chandra, R. and Kaushik, S. (2003). Studies on anti diarhheacl activity of calotropies Gigantea

R. Br. In experimental, Animals. Journal of pharmacy and pharmaceutical sciences (7): pp. 70-75.
Elamin, H. M. (1990). Trees and shrumbs of the Sudan. Ithaca press, exeter.
Elkamali, H. M. and Elkhalifa, K. F. (1997). Treatment of malaria through herbal drugs in the central Sudan. Fitoterapia 68, pp. 527 – 528.
Fabrega, H. and Silver D. (1973). Illness and shamanistic curing in zinacantan an ethnomedical analysis.University press, standford.
Farnsworth, N. R.; akerele, O.; Bingel, A.s.; Soejarto, D.D. and Guo, Z. Bull. WHO.(1985) 63, pp. 965-972.
Forbes, B.A.; Saham, D.F.; Wiessfied,A.S. and Trevino, E.A. (1990). Methods for testing antimicrobial effectiveness In: Bailey and Scotts Diagnostic Microbiology Ed. Baron E.J., Peterson L.R. and fine gold S. m. Mosby Co. St Louis, Missouri.
Ghosh, A; Das, B; Roy, A; M\andal, B. and Chandra, G. (2008).Anti-bacterial activity of some medicinal plant extracts. Journal of natural medicines, 62, pp. 259 – 262.
Harborne, J. B. (1998). Phytochemical methods. Halsted press. New York.
HILL AF. (1952). Economic Botary a text book of useful plants and plants products 2nd ed McGraw-Hill Book Company Inc New York.
Igoli, J. O; I. C. Igwue and N. P. Igoli (2003). Traditional medicinal practices amongst the igode people of Nigeria, J. herbs spices and medicinal plants, 10 (4), pp. 1-10.
Iwu MM,Duncan AR and Okunji CO. (1999). New antimicrobials of plant origin. In: Janick J. ed. Prospective on new crops and new uses. ASHS press, Alexandria, V.A.pp.457-462.
Kim, H. (2005). Do not put too much value on conventional medicines. Journal of ethnoharmacolgy, 37, 39, 100.
Raskin,I.:Ribnicky,D.:Komarnytsky,S.:IIic,N.:Poulev,A.:Borisiuk,N.,
Brinker, A.: Moreno, A.: Ripoll, C.: Yakoby, N.: cornwell, T.: Pastor,I. and Fridlender B. (2002). Plant and the human health in the twenty-firest century,trends in Biotechnology, 20(12), pp.522-531.
Shimada k, Fujikawa k, Yahara k, and Nakamura T. (1992). Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. Agric Food Chem.: 40: pp. 945-8.
Sofowora A. (1982). Medicinal Plants and Traditional in Afirica. John Willy, New York, pp. 289-290.
Solecki, R.S., (1975). Shanidar IV: a Neanderthal flower burial in northern Iraq. Science 190, 880–81.
Westh H, Zinn CS, Rosdahl VT. (2004). An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aurous isolated from 15 hospitals in 14 countries. Microbe Drug Resist. (10) pp.169-176