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DNA Fingerprinting, Chemical Composition and Antimicrobial Activity of the Essential Oil isolated from the Fruits of *Serenoa repens* W. Bartram

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Abstract

The chemical composition of the essential oil obtained from the fruits of *Serenoa repens* W. Bartram by hydrodistillation has been studied by GC/MS analysis. The analysis of the essential oil revealed the presence of 60 identified compounds (monoterpene and sesquiterpene) oxygenated and non-oxygenated compounds in the oil sample including about **53** compounds which were not reported in GC/MS analysis of the essential oil before. GC/MS analysis revealed 4-(1-methylethyl)-Benzaldehyde to be the major constituent of the essential oil of the fruits 58.56% followed by 2-Caren-10-al and 3-Caren-10-al (11.83% and 2.87%, respectively).

So far nothing could be traced concerning the oil biological activity. The antimicrobial sensitivity as well as the MIC against different fungal, gram positive and gram negative strains was carried out. The antimicrobial snsitivity was higher as antifungal followed by the Gram-positive strains, and Gram-negative bacteria strains compared to the positive controls. The essential oil showed high selective antimicrobial potential (MIC 1.95–62.5 µg/mL for bacteria; and MIC 3.9–31.25 µg/mL for fungi). DNA fingerprinting of the cultivated leaves were carried out for authentication of the plant.

Keywords: *Serenoa repens*, essential oil, 4-(1-methylethyl)-Benzaldehyde, Antimicrobial, GC/MS, DNA.

1. Introduction

Serenoa repens W. Bartram, common name was saw palmetto, was considered the sole representative of Serenoa family Arecaceae (Morabito et al., 2015). It was also called *Serenoa serrulata* Michaux and *Sabal serrulata* Michx. (Lim, 2012). It was found growing in the Southeastern part of the United States, especially in South Carolina, Florida, and Southern California and in the West India (Allkanjari &Vitalone, 2015).

The fruit part was used since decades in the treatment of infertility and impotence. It was firstly reported in the treatment of urinary complaints from the beginning of the 20th century (Laekeman & Vlietinck, 2013). The fruits were used for the treatment of prostate inflammation and benign prostatic hyperplasia (Ross, 2005).

Recently the volatile oil was isolated from the fruits (Khwaja & Friedman, 2000) and the chemical composition of the hydrodistillate formed by steam distillation was analyzed by GC/MS to identify 144 steam-volatile constituents (Roesler et al., 2009).

It was found that the combination of broad spectrum short-lasting antibiotic therapy with *Serenoa repens W. Bartram* showed better control and recurrence rate on patients with chronic bacterial prostatitis with Gram-negative uropathogens as *Escherichia coli* and *Enterobacter* spp. than antibiotic treatment alone (Busetto et al., 2014). So far nothing could be traced concerning the volatile oil of the cultivated Indian one or its antimicrobial activity. It was interesting to focous in this paper on the volatile oil analysis as well as determination of its antimicrobial activity.

2. Material and methods

2.1. Plant material

The fruits in this study were supplied by Haraz, Cairo, Egypt and they were originated from India and then identified and conducted by Prof. Dr. Abdel-Halim Mohammed; Professor of Agriculture, Flora department, Agricultural museum, Cairo, Egypt. Voucher speciemen were kept in the Agricultural museum.

2.2. DNA fingerprinting:

2.2.1. Materials for DNA fingerprinting

Sample from the cultivated plant was used and saved in ice box and quickly transported to laboratory. Whole fresh leaves were freeze dried and ground to a fine powder under liquid nitrogen.

2.2.2. DNA extraction

DNA isolation was performed using the CTAB method (cetyltrimethyl ammonium bromide) of (Doyle, 1990). 0.5 g of fresh leaves was powdered in liquid nitrogen, suspended in 1 ml preheated (65°C) CTAB buffer, incubated at 65°C for 1 hour with occasional shaking, and then centrifuged for 15 minutes at 1000 rpm. The supernatant was transferred to a new tube by wide pore, 0.5 ml of chloroform and isoamyl alcohol mixture (24:1) was added forming an emulsion which is centrifuged for 15 minutes at 14000 rpm and aqueous layer was transferred to a new sterilized tube (to avoid protein surface). Ice-cold isopropanol was added to precipitate the nucleic acid (RNA, DNA), incubated at –20°C overnight, and then centrifuged at 14000 rpm for 20 minutes. The supernatant was discarded and the nucleic acid pellet was washed carefully twice with cold 70% ethanol, and resuspended in 100 µl of TE buffer.

2.2.3. DNA amplification

RAPD analysis (Rapid Amplified Polymorphic DNA) was performed as described by (Williams et al., 1990) with minor modifications, using PCR technique (Polymerase Chain Reaction) to amplify nanograms of the total genomic DNA and by using ten different random primers (A1, A2, A3, A4, A5, A6, A7, A8, A9 and A10) with sequences showed in table (1). Briefly, PCR amplification was performed with 25 µl reaction mixture containing the following reagents: 0.5 µl of dNTPs (10 mM), 1.5 µl $MgCl_2$, 5 µl of 10 x PCR buffer, 2.0 µl of primer (5 p mol), 2.0 µl of total genomic DNA (20.40 ng), 0.25 µl of Taq polymerase (0.5 unit) and 14.75 µl of sterile double distilled H_2O. The reaction was assembled on ice and overlaid with a drop of mineral oil. The DNA amplifications were performed in an automated thermal cycle programmed for one cycle at 95^oC for 3 min followed by 45 cycles of 2 min at 92^oC, 1 min at 37^oC and 2 min at 72^oC. The reaction was finally stored at 72^oC for 10 min then incubated at 4^oC.

2.2.4. Agarose electrophoresis

Each PCR-amplification product was analyzed by electrophoresis technique using 2% agarose gel in TAE buffer stained with 0.2 µg/ml ethidium bromide (to enable visualizing the obtained RAPD pattern to be photographed later under UV light). TAE buffer was added to the agarose, heated in a microwave till melting, cooled to 60°C, and then ethidium bromide was added. Sample, loaded on agarose gel, was prepared by mixing 10 µl PCR products with 2 µl loading buffer. The gels were run at 100 volts for approximately 30 minutes. One marker; Axygen, was used (100 bp DNA ladder). RAPD pattern was photographed under UV light figure (1).

2.3. Investigation of the volatile constituents

2.3.1. Volatile oil extraction

Half-kilo of the fruits of *Serenoa repens* W. Bartram was crushed and placed in a 4 liters flask with 1.5 liter of water. The mixture was subjected to hydrodistillation using a Clevenger type apparatus for 6 hrs. The volatile fraction was extracted by diethyl ether (20 ml). This extract was dried over anhydrous sodium sulphate (Egyptian Pharmacopoeia, 2005). It yielded about 0.5 ml yellowish, aromatic-smelling oil. The oil was stored at low temperature till analysis.

2.3.2. GC/MS analysis of the volatile oil of the fruits of *Serenoa repens* W. Bartram

The oil analysis was performed on a Hewlett– Packard-5 MS 6890N Agilent gas chromatogram equipped with Agilent mass spectrophotometric detector, with a direct capillary interface and fused silica capillary column DB-5 (5% phenyl methyl polysiloxane) stationary phase, (30 m x 320 µm x 0.25 µm film thickness), helium as carrier gas with flow rate 1 ml/min. Oven temperature was 40^oC and was programmed to 280^oC at a rate of 2^oC /min., while the injector temperature was 250^oC and detector temperature was 280^oC. Injection volume was 1µl and splitless mode.

Mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV scanning from 50 to 900 m/z. The volatile compounds of the oil were identified by their retention indices (RI, determined with reference to a homologous series of normal alkanes), and by comparing their mass fragmentation patterns with those of the available reference (Sparkman, 2005). Also, compound identification was confirmed by MS libraries [NIST 05; Mass Finder database (G1036A, revision D.01.00 and Wiley7 Mass Finder].

2.4. Determination of antimicrobial activity

2.4.1. Microorganisms for antimicrobial activity

The following available stocks in the Micro Analytical Center, AL-Azhar University, Cairo, Egypt, were used; Gram-positive bacteria as (*Bacillus subtilis* (RCMB 010067), *Streptococcus pneumoniae* (RCMB 010010) and *Staphyllococcus aureus* (RCMB 010028)), Gram-negative bacteria as (*Pseudomonas aeruginosa* (RCMB 010043), *Salmonella typhimurium* (RCMB 010072), *Klebsiella oxytoca* (RCMB 01002 83-4) and *Escherichia coli* (RCMB 010052)), and Fungi as (*Candida albicans* (RCMB 05031), *Penicillium italicum* (RCMB), *Fusarium oxysporum* (RCMB), *Fusarium s. cucurbitae* (RCMB) and *Aspergillus fumigatus* (RCMB 02568)).

2.4.2. Culture media and antibiotics for antimicrobial assay

Brain Heart Infusion as liquid and solid media (HiMedia), Mueller-Hinton agar (HiMedia), that was rigorously tested for composition and pH, and Potato Dextrose Agar medium (HiMedia) were used. Ampicillin (Oxoid, UK) was used as standard antibacterial agent for Gram positive bacteria while Gentamycin (Oxoid, UK) was used as standard antibacterial agent for Gram negative bacteria also Amphotericin B (Sigma Chemical Co., St. Louis, Mo.) was used as a standard antifungal agent.

2.4.3. Procedure

The antimicrobial activity of the extract was screened by the agar disc diffusion method with slight modification (Sahoo et al., 2006; NCCLS, 1999). The bacterial cultures were grown in Brain Heart Infusion broth at 37°C. After 6 hrs of growth, 100 µl of each microorganism at a concentration of $1x10^6$ cells/ml was inoculated on the surface of Mueller-Hinton agar plates for bacteria and Potato Dextrose Agar plates for fungi.

DMSO with a concentration up to 2% was used to dissolve the extract. Filter paper discs (6 mm in diameter) saturated with 20 µL of the tested oil extract or DMSO (solvent control) were placed on the surface of the inoculated plates. To evaluate the efficiency of the methodology; 50 µl of the extract was inserted simultaneously in a hole made in new plates. The plates were incubated at 37°C for 24 hrs. The diameter of the inhibition zone was measured in millimeter, and was recorded as mean ± standard deviation (SD) of a triplicate experiment. Antimicrobial activity of the investigated volatile oil determined as the cultured species producing halos equal to or greater than 7 mm were considered susceptible to the tested samples. Ampicillin (10 µg) and Gentamicin (10 µg) discs were used for bacteria samples as positive standard and Amphotericin B (5 µg) was used as positive standard for fungi.

2.4.4. Determination of minimum inhibitory concentration assay

The broth micro-dilution technique was used to determine the MIC values. All of the experiments were performed in Mueller Hinton broth (Hi Media, Mumbai) for the bacterial strains and RPMI 1640 medium for the fungal strain. Two-fold serial dilution of the volatile oils was prepared in a 96-well microtiter plate up to 2 mg/ml. The prepared microtiter plates containing the microorganisms and the volatile oils were then incubated at 37°C for 24 hrs for bacterial growth and at 27°C for 48 hrs for fungal growth. The growth of organisms was observed as turbidity, which was visually observed. Controls were set up with equivalent quantities of dimethyl sulfoxide 10% solution, which was used as a solvent for the volatile oils (NCCLS, 1999). Amoxicillin, Gentamicin and Amphotericin B (Sigma, USA) were used as positive controls.

3. Results and Discussion

3.1. DNA fingerprinting

The RAPD electrophoretic profile of the DNA sample of *Serenoa repens* W. Bartram amplified with the ten random primers (A1, A2, A3, A4, A5, A6, A7, A8, A9 and A10 respectively) showed distinguishable bands generating 120 fragments. The distribution of these bands is illustrated in figure (1) and table (2). Each of the ten primers successfully directed the amplification of a genome-specific fingerprint of DNA fragments; all amplifications were found to be useful.

A total of 120 different fragments have been recorded, produced by (A01) showing 11 bands ranging from 1481 bp to 294 bp, (A02) showing 12 bands ranging from 1481 bp to 250 bp, (A03) showing 10 bands ranging from 1481 bp to 294 bp, (A04) showing 12 bands ranging from 1481 bp to 250 bp, (A05) showing 12 bands ranging from 1481 bp to 250 bp, (A06) showing 13 bands ranging from 1481 bp to 250 bp, (A07) showing 13 bands ranging from 1481 bp to 250 bp, (A08) showing 13 bands ranging from 1481 bp to 250 bp, (A09) showing 13 bands ranging from 1481 bp to 250 bp, (A10) showing 11 bands ranging from 1481 bp to 250 bp. The estimated RAPD-primers, which produce high values of similarity coefficient and low levels of polymorphism, could be used in the identification of the plant.

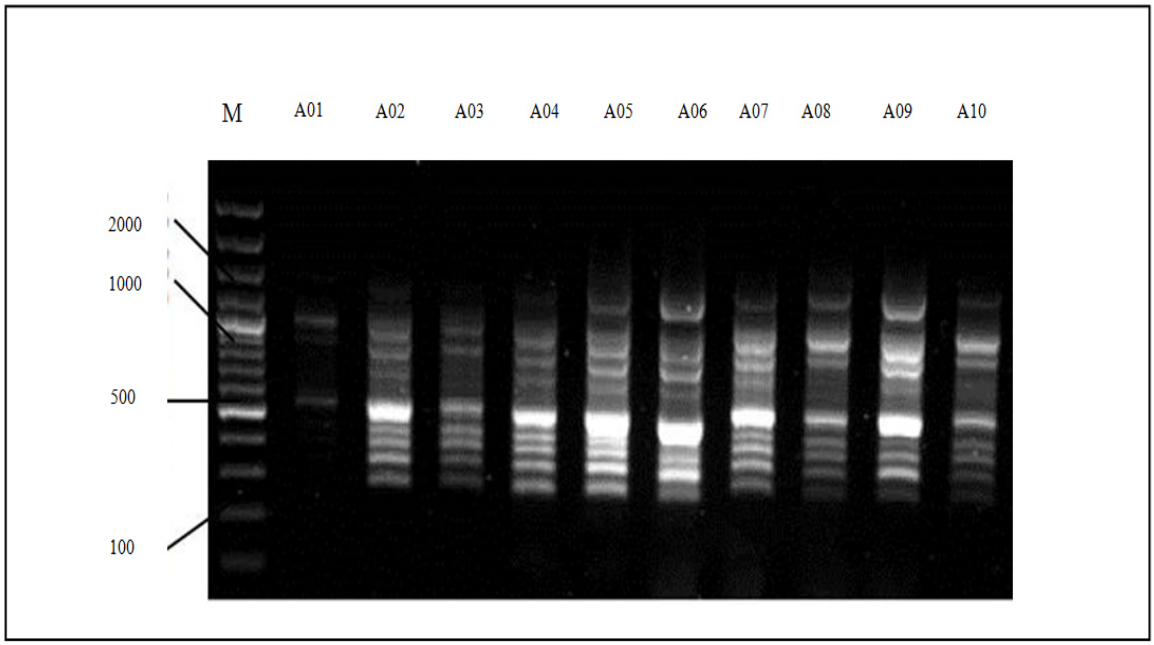


Figure (1): RAPD electrophoretic profile of *Serenoa repens* W. Bartram generated by ten primers.

Table (1): Name and sequence of the selected random primers used in RAPD-PCR analysis.

Order	Primer code	Nucleotide sequences (5`- 3`)
1	A1	CAGGCCCTTC
2	A2	TGCCGAGCTG
3	A3	AGTCAGCCAC
4	A4	AATCGGGCTG
5	A5	AGGGGTCTTG
6	A6	GGTCCCTGAC
7	A7	GAAACGGGTG
8	A8	GTGACGTAGG
9	A9	GGGTAACGCC
10	A10	GTGATCGCAG

Table (2): RAPD polymorphic bands of *Serenoa repens* W. Bartram produced by ten primers.

Bands bp	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10
1481	+	+	+	+	+	+	+	+	+	+
1194	+	+	+	+	+	+	+	+	+	+
1026	+	+	+	+	+	+	+	+	+	+
907	+	+	+	+	+	+	+	+	+	+
808	+	+	+	+	+	+	+	+	+	+
659	+	+	+	+	+	+	+	+	+	+
546	+	-	-	-	-	+	+	+	+	-
513	-	+	+	+	+	+	+	+	+	-
464	+	+	-	+	+	+	+	+	+	+
389	+	+	+	+	+	+	+	+	+	+
347	+	+	+	+	+	+	+	+	+	+
294	+	+	+	+	+	+	+	+	+	+
250	-	+	-	+	+	+	+	+	+	+
Total	**11**	**12**	**10**	**12**	**12**	**13**	**13**	**13**	**13**	**11**

3.2. Composition of the volatile oils

The GC/MS analysis of the hydrodistillated volatile constituents from the fruits of *Serenoa repens* W. Bartram originated from India result in identification of 60 compounds including (monoterpene and sesquiterpene) oxygenated and non-oxygenated compounds, the relative concentrations were presented in figure (2 and 3) and table (3).

Approximately 76.1% of the hydrodistillate oil was oxygenated compounds (15 compounds), of which about 12 monoterpens where 4-(1-methylethyl)-Benzaldehyde (58.56%) was found to be the major constituent of the oil followed by 2-Caren-10-al (11.83%) and 3 sesquiterpenes wherein α-Chamigrene (0.14) was the major compound on the other hand previously reported that the main component of the hydrodistillate originated from North America was lauric acid (40.4%) (Roesler et al., 2009).

Furthermore, 23.9% of the hydrodistillate was non-oxygenated (45 compounds) formed by a group of 11 monoterpenes wherein γ-Terpinene (0.67%) showed the highest percentages, 34 sesquiterpenes identified where trans-Caryophyllene (2.87%) was the major one differ from previously reported α-copaene (0.1%) (Roesler et al., 2009).

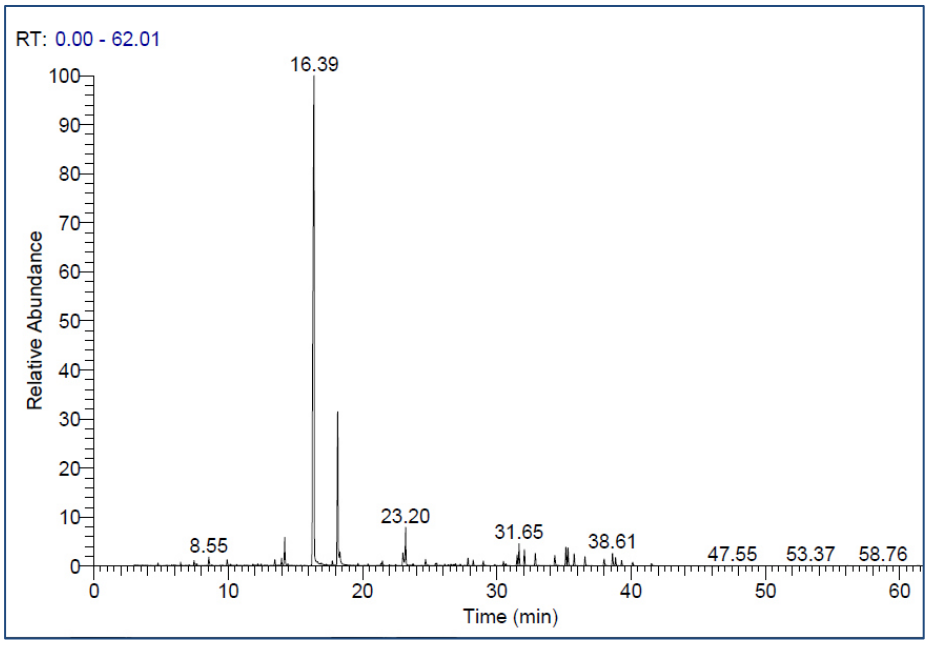


Figure (2): Total ion chromatogram of GC/MS analysis of the hydrodistilled volatile oil of the fruits of *Serenoa repens* W. Bartram.

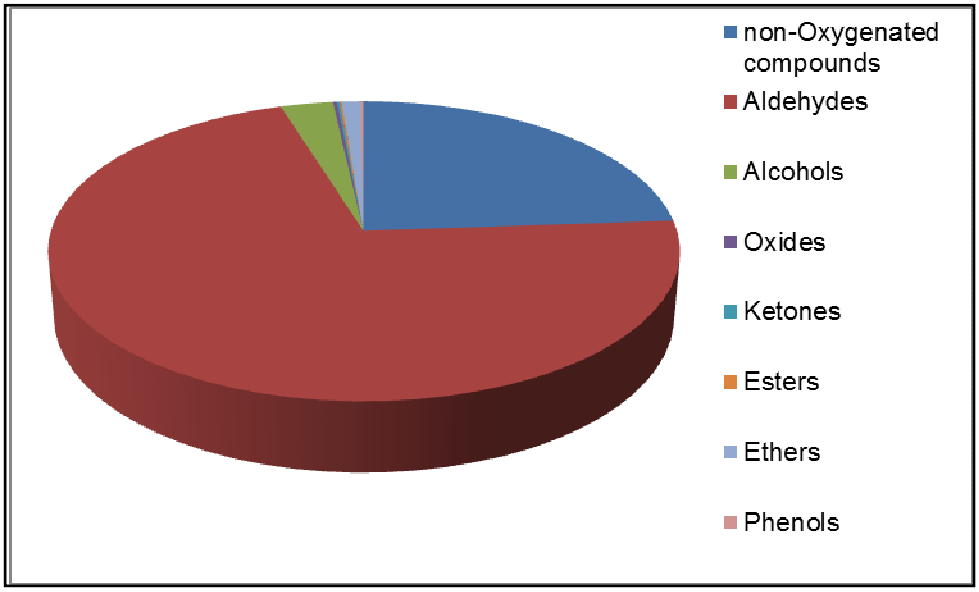
Table (3): compounds identified by GC/MS analysis of the hydrodistilled volatile oil of the fruits of *Serenoa repens* W. Bartram.

Peak no.	Identified compound	kI	RT	RRT	M.Wt	Base peak	Fragments	Area %
1	(-)-α-Pinene	939	4.77	0.29	136	93	77, 105, 67, 136	0.14
2	Decane	999	6.45	0.39	142	57	97, 142, 71, 85	0.25
3	2,6-dimethyl-Nonane	1026	7.09	0.43	156	71	57, 85, 113, 97	0.09
4	p-Cymene	1026	7.45	0.45	134	119	77, 91, 103, 134	0.34
5	R-(+)-Limonene	1031	7.53	0.46	136	119	68, 93, 79, 134	0.09
6	(1-methyl propyl)-Cyclohexane	1032	7.59	0.463	140	83	55, 67, 119, 140	0.09
7	Eucalyptol	1033	7.67	0.47	154	81	71, 93, 108, 55	0.13
8	γ-Terpinene	1062	8.55	0.52	136	93	77, 136, 121, 57	0.67
9	Undecane	1099	9.91	0.6	156	57	71, 85, 98, 156	0.44
10	L-Linalool	1098	10.17	0.62	154	71	93, 55, 80, 121	0.15
11	trans-Pinocarveol	1139	11.84	0.72	152	92	55, 70, 119, 134	0.12
12	(+)-2-Bornanone	1143	12.20	0.74	152	95	81, 69, 108, 152	0.15
13	2-methyl –Undecane	1171	12.45	0.76	170	57	71, 85, 99, 126	0.12
14	Terpinen-4-ol	1177	13.47	0.82	154	71	55, 93, 111, 136	0.45
15	4-(1-methylethyl)-1,3-Cyclohexadiene-1-methanol	1194	13.97	0.85	152	81	109, 67, 91, 121	2.22
16	Dodecane	1199	14.20	0.86	170	57	71, 85, 98, 127	0.53
17	2,6-dimethyl- Undecane	1210	14.44	0.88	184	57	71, 98, 133	0.10
18	4-(1-methylethyl)-Benzaldehyde	1239	16.38	1	148	133	105, 148, 77, 117	58.56
19	Phellandral	1252	17.75	1.08	152	109	67, 79, 55, 121	0.35
20	2-Caren-10-al	1289	18.14	1.11	150	79	107, 91, 121, 135	11.83
21	3-Caren-10-al	1311	18.31	1.12	150	107	79, 91, 121, 65	0.53

22	δ-Elemene	1339	19.66	1.2	204	121	93, 136, 161, 77	0.16
23	α-Terpinyl acetate	1350	20.41	1.24	196	121	93, 136, 79, 107	0.12
24	β-Elemene	1375	21.37	1.3	204	93	81, 67, 107, 147	0.08
25	α-Copaene	1376	21.99	1.34	204	105	161, 93, 81, 55	0.16
26	Tetradecane	1399	22.46	1.37	198	57	71, 85, 99, 113	0.10
27	Methyleugenol	1401	23.00	1.4	178	91	147, 77, 65, 135	1.12
28	trans-Caryophyllene	1408	23.20	1.42	204	91	69, 79, 133, 105	2.87
29	Calarene	1432	23.47	1.43	204	161	121, 93, 79, 133	0.41
30	trans-α-Bergamotene	1436	23.76	1.45	204	119	93, 69, 107, 55	0.15
31	(E)-β-Farnesene	1458	24.69	1.5	204	93	69, 79, 133, 55	0.56
32	δ - muurolene	1467	25.44	1.55	204	161	105, 120, 79, 133	0.17
33	β-Acoradiene	1473	25.52	1.56	204	119	93, 105, 79, 67	0.19
34	β-Selinene	1485	26.12	1.59	204	105	93, 67, 121, 161	0.12
35	α-Selinene	1494	26.38	1.61	204	189	93, 107, 133, 81	0.09
36	α-Chamigrene	1500	26.58	1.62	204	57	93, 71, 121, 107	0.14
37	butyl hydroxyl Toluene	1508	26.75	1.63	220	205	57, 91, 177, 119	0.14
38	β-Bisabolene	1509	26.92	1.64	204	93	69, 79, 107, 55	0.16
39	δ-Cadinene	1524	27.28	1.66	204	161	119, 105, 134, 91	0.12
40	(1-butyl hexyl)-Benzene	1526	27.85	1.7	218	91	105, 147, 119	0.62
41	(1-propyl heptyl)-Benzene	1538	28.23	1.72	218	91	113, 105, 119	0.47
42	(1-ethyl octyl)-Benzene	1560	28.99	1.77	218	91	119, 105, 189	0.37
43	(-)-Caryophyllene oxide	1581	29.84	1.8	220	91	69, 55, 107, 133	0.11
44	(1-methyl nonyl)-Benzene	1593	30.49	1.86	218	105	91, 79, 57	0.37
45	Carotol	1594	30.67	1.87	222	161	105, 69, 81, 119	0.13
46	(1-pentyl hexyl)-Benzene	1625	31.51	1.92	232	91	119, 105, 161	0.79
47	(1-butylheptyl)-Benzene	1628	31.65	1.93	232	91	105, 147, 175	1.71
48	(1-propyloctyl)-Benzene	1638	32.05	1.95	232	91	133, 105, 189	1.30
49	(1-ethylnonyl)-Benzene	1659	32.86	2	232	91	119, 105, 203	1.05
50	(1-methyldecyl)-Benzene	1696	34.32	2.1	232	105	91, 79, 119	0.86
51	(1-pentylheptyl)-Benzene	1718	35.14	2.14	246	91	105, 119, 161	1.45
52	(1-butyloctyl)-Benzene	1723	35.30	2.15	246	91	105, 147, 189	1.40
53	(1-propylnonyl)-Benzene	1734	35.75	2.18	246	91	133, 105, 203	1.01
54	(1-ethyldecyl)-Benzene	1758	36.56	2.23	246	91	105, 133, 119	0.78
55	(1-methylundecyl)-Benzene	1797	37.99	2.31	246	105	91, 79, 119	0.58
56	(1-pentyloctyl)-Benzene	1819	38.61	2.36	260	91	105, 119, 161	1.12
57	(1-butylnonyl)-Benzene	1821	38.84	2.37	260	91	105, 147, 203	0.69
58	(1-propyldecyl)-Benzene	1838	39.29	2.39	260	91	133, 105, 217	0.46
59	(1-ethylundecyl)-Benzene	1866	40.11	2.44	260	91	119, 105, 231	0.33
60	(1-methyldodecyl)-Benzene	1906	41.51	2.53	260	105	91, 79, 55	0.20
Total identified component %					**100%**			
Oxygenated compounds					76.1%			
non-Oxygenated compounds					23.9%			
Monoterpenes					78.57%			
Sesquiterpenes					21.43%			
Aldehydes					71.26%			
Alcohols					3.07%			
Oxides					0.24%			
Ketones					0.15%			
Esters					0.12%			
Ethers					1.12%			
Phenols					0.14%			

kI = retention index as determined on DB -5 column based on a homologous series of normal alkanes and by using (Adams, 2007), RRT= relative retention time which is relative to 4-(1-methylethyl)- Benzaldehyde (16.38), Structure confirmed by (Willey7N; Mass Finder and Willey 7 Nist 05; Mass Finder) libraries without determination of their retention indices due to no available standard compound in retention index libraries.

Figure (3): The calculated percentage of different classes of components of essential oil from the fruits of *Serenoa repens* W. Bartram



3.3. ***Antimicrobial activity***

The antimicrobial activity of the investigated oils was evaluated by determiniation of the antimicrobial sensitivity and MIC values figure (4) and tables (4, 5) against three Gram-positive and four Gram-negative bacteria as well as five fungal strains. The results showed that the oil had moderate antimicrobial activities against different fungal and bacterial strains. The activity was higher as antifungal followed by the Gram-positive strains, Gram-negative bacteria strains compared to the positive controls, as shown in table (4). The MIC was determined against selected strains. The oil was active as antifungal against *Fusarium s. cucurbitae* (RCMB) with MIC 3.9 µg/ml, followed by *Fusarium oxysporum* (RCMB) with concentration 7.81 µg/ml. It showed a moderate antibacterial activity against *Bacillis subtilis* (RCMB 010067), Gram positive, with concentration 1.95 µg/ml on the other hand the other fungal and bacterial strains were poorly active or inactive compared to the positive controls. These activities may be attributed to the unique chemical composition of the volatile oil and the presence of the major isolate 4-(1-methylethyl)-benzaldehyde that had strong fungistatic activity as reported in previous studies (Zhai et al., 2011; De et al., 2003).

Table (4): Antimicrobial activity of volatile oil extract of *Serenoa repens* W. Bartram

Sample / **Tested microorganisms**	**Volatile oil**	**Standard .**
FUNGI		***Amphotericin B***
Aspergillus fumigatus (RCMB 02568)	16.3 ± 1.2	23.7± 0.10
Penicillium italicum (RCMB)	NA	21.9± 0.12
Candida albicans (RCMB 05031)	NA	19.8± 0.20
Fusarium oxysporum (RCMB)	18.2 ± 0.72	25.4± 0.16
Fusarium s. cucurbitae (RCMB)	20.3 ± 0.63	26.7± 0.13
Gram Positive Bacteria		***Ampicillin***
Staphylococcus aureus (RCMB 010028)	14.2 ± 0.58	27.4± 0.18
Streptococcus pneumoniae (RCMB 010010)	NA	20.3± 0.58

Bacillis subtilis (RCMB 010067)	21.4 ± 0.72	32.4± 0.3
Gram negativeBacteria		***Gentamicin***
Pseudomonas aeruginosa (RCMB 010043)	NA	17.3± 0.15
Salmonella typhimurium (RCMB 010072)	16.2 ± 0.63	28.8± 0.24
Escherichia coli (RCMB 010052)	15.4 ± 1.2	22.3± 0.18
Klebsiella oxytoca (RCMB 01002 83-4)	NA	25.3± 0.63

NA (no activity)

Table (5): Antimicrobial Activity as MICS (µg/ml) of the volatile oil against tested microorganisms

Sample / Tested microorganisms	**Volatile oil**	**Standard**
FUNGI		**Amphotericin B**
Aspergillus fumigatus (RCMB 02568)	31.25	0.98
Penicillium italicum (RCMB)	NA	1.95
Candida albicans (RCMB 05031)	NA	3.9
Fusarium oxysporum (RCMB)	7.81	0.98
Fusarium s. cucurbitae (RCMB)	3.9	0.98
Gram Positive Bacteria		***Ampicillin***
Staphylococcus aureus (RCMB 010028)	62.5	0.98
Streptococcus pneumoniae (RCMB 010010)	NA	3.9
Bacillis subtilis (RCMB 010067)	1.95	0.98
Gram negativeBacteria		***Gentamicin***
Pseudomonas aeruginosa (RCMB 010043)	NA	15.63
Salmonella typhimurium (RCMB 010072)	31.25	0.98
Escherichia coli (RCMB 010052)	62.5	1.95
Klebsiella oxytoca (RCMB 01002 83-4)	NA	0.98

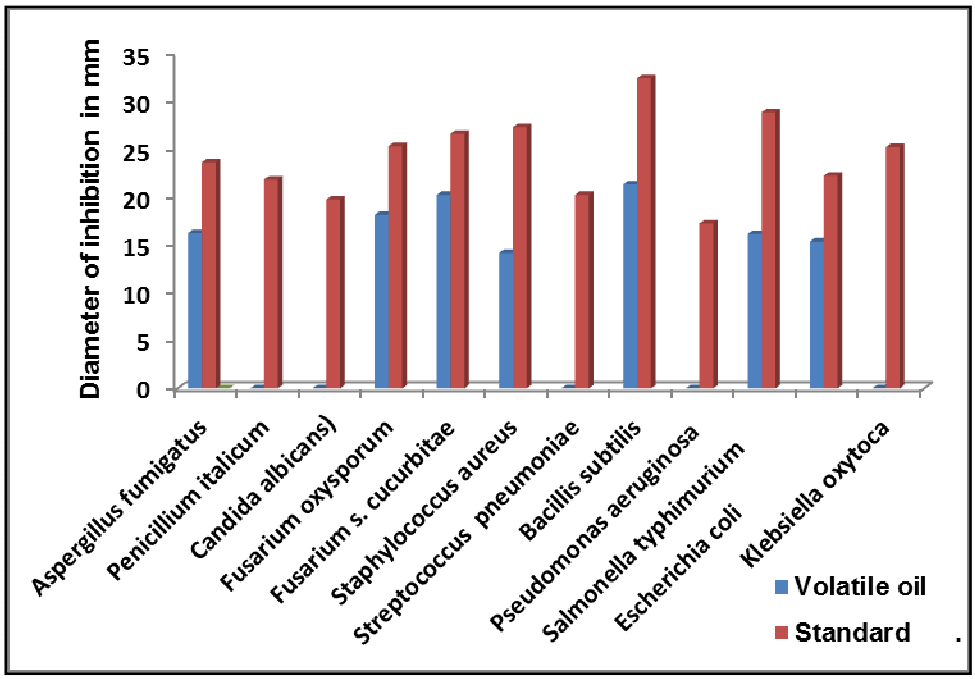


Figure (4): Antimicrobial activity of volatile oil extract of *Serenoa repens* W. Bartram against certain microorganisms

Conclusion

To the best of our knowledge, this is the first report on either the chemical composition or antimicrobial activity of the volatile oil the fruits of *Serenoa repens W. Bartram*. The analysis of the volatile constituents revealed the presence of a number of identified (monoterpene and sesquiterpene) oxygenated and non-oxygenated compounds in the oil sample. These volatile compounds exhibited different degrees of antimicrobial activity against Gram positive and Gram negative bacteria and fungi. These results may direct to future work for testing the volatile oil against other microbial strains as well as figuring out its mechanism of action.

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