



Trends in Phytochemical Research (TPR)

Journal Homepage: <http://tpr.iau-shahrood.ac.ir>



Original Research Article

Volatile oil composition, antioxidant and antimicrobial properties of *Boerhavia erecta* L. and *Euphorbia hirta* L.

OMOSALEWA OLAOLUWA ✉, DORCAS MORONKOLA, OLAYOMBO TAIWO AND PEREMOBOWEI IGANBOH

Department of Chemistry, Faculty of Science, University of Ibadan, 200284, Ibadan, Nigeria

ABSTRACT

This study investigated the volatile oil composition of *Boerhavia erecta* L. and *Euphorbia hirta* L. along with the corresponding antioxidant and antimicrobial properties. In this regard, stems and roots of *Boerhavia erecta* together with aerial parts of *Euphorbia hirta* L. were used. Essential oils were obtained by hydrodistillation method using Clevenger-type apparatus, while identification and characterization were done by gas chromatography-mass spectrometry (GC-MS) technique. Antioxidant activities were evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) method and the antimicrobial properties were assessed by agar diffusion method. GC-MS analyses revealed seven and twenty-four components in stem and root oils of *Boerhavia erecta* L., respectively representing 81.84% and 76.93% of the total oil contents with phytol (61.31%) and 9-octadecenamide (33.95%) as the most abundant components, respectively. Eleven compounds were identified in *E. hirta* L. oil representing 92.79% of the oil. The major compound of this essential oil was (Z)-9-octadecenamide (60.71%). The other minor components were methyl hexadecanoate (7.02%) and phytol (4.80%). *B. erecta* and *E. hirta* oils showed significant antioxidant activities compared to α -tocopherol. *B. erecta* L. stem oil inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* bacterial strains, while root oil showed moderate inhibition against *Salmonella typhi* and *Bacillus subtilis* at inhibition zones of 14-20 mm over a concentration of 12.5-50%. The three oil samples displayed significant antifungal activities against *Candida albicans*. Chemical constituents of *B. erecta* L. and *E. hirta* L. oils may be responsible for these biological activities. These evaluations are reported for the first time.

© 2018 Islamic Azad University, Shahrood Branch Press, All rights reserved.

ARTICLE HISTORY

Received: 03 July 2018
Revised: 14 August 2018
Accepted: 22 September 2018
ePublished: 27 September 2018

KEYWORDS

Volatile oil
Gas chromatography-mass spectrometry (GC-MS)
Phytol
Biological activities

1. Introduction

Since the ancient times, medicinal plants have been used in virtually all cultures as a source of medicine for the treatment of a broad spectrum of diseases throughout the world (Nunes and Miguel, 2017). The World Health Organization (WHO) reported that 80% of the emerging world's population relies on traditional medicine for therapeutic purposes. Essential oils are naturally found in various medicinal plants with a characteristic pungent odor and usually have a lower density as compared to water (Mohammedhosseini et al., 2013). Previous scientific studies clearly revealed that essential oils exhibit pharmacological properties involving antiplasmodial, antioxidant, antimicrobial, antiviral, antimutagenic, anticancer, insecticidal, anti-

inflammatory and immunomodulatory activities (Camilo et al., 2017). In fact, the phytochemical constituents present in the essential oils are responsible for their various therapeutic virtues (Mohammedhosseini et al., 2017).

Boerhavia erecta L. is a medicinal weed of the family of Nyctaginaceae commonly known as 'Hogweed'. The genus *Boerhavia* is widely distributed in tropical, subtropical and temperate regions of the world including Mexico, America, Africa, Asia, Pacific Islands and Australia. *B. erecta* L. has been widely used in traditional medicine for the treatment of various ailments such as hepatitis, urinary disorders, gastrointestinal diseases, inflammations, skin problems, infectious diseases and asthma (Patil and Bhalsing, 2016). It has been reported to possess anti-inflammatory, anti-

✉ Corresponding author: Omosalewa Olaoluwa
Tel: +2349032075420; Fax: + 2349032075420
E-mail address: omosalewa.olaoluwa@gmail.com



convulsant, antifibrinolytic, hepatoprotective, antimicrobial (Suriyavathana et al., 2012), antioxidant and anticancer properties (Shareef et al., 2017).

Compounds such as 3',4',5,7-tetrahydroxyflavone-3-O- α -D-rhamnopyranosyl-(1 \rightarrow 6)-O- β -D-glucopyranoside; 4',5,7-tetrahydroxy-3'-methoxyflavone-3-O- α -D-rhamnopyranosyl-(1 \rightarrow 6)-O- β -D-glucopyranoside (Nugraha, 2009) and 2,3-dihydroxypropylbenzoate 3-O- β -[4"- methoxy] glucuronide have been isolated from *B. erecta* L. (Nugraha et al., 2015). The betacyanin compounds such as betanin, isobetainin and neobetainin were also found in the stem bark of *B. erecta* L. (Stintzing et al., 2004; Calderón-Montaño et al., 2011).

Euphorbia hirta L. belongs to the family Euphorbiaceae, the spurge family, which occurs mainly in the tropics, with the majority of the species found in the Indo-Malayan region and tropical America (Aworinde et al., 2009). *E. hirta* L. is used traditionally in the treatment of conjunctivitis, gastrointestinal disorders, bronchial and respiratory diseases. It is used as an antidote and an effective pain relief against scorpion and snake bites. The stem sap is used in the treatment of eyelid sty, swelling and boils. *E. hirta* L. was found to exhibit anxiolytic, analgesic, anticancer, antipyretic, larvicidal, antioxidant, anthelmintic, antimicrobial and anti-inflammatory activities (Adedapo et al., 2005; Sharma et al., 2009). The aqueous extract of the herb strongly inhibited aflatoxin contamination in rice, wheat, maize and mustard crops (Kumar et al., 2010a; Rao et al., 2010). However, *E. hirta* L. was found to be highly toxic (Adedapo et al., 2003).

In previous attempts, the methanol extract of *E. hirta* L. has been reported to possess afzelin, quercitrin, myricitrin, rutin, gallic acid, quercetin, euphorbin-A and euphorbin-B, euphorbin-C, euphorbin-D, 2,4,6-tri-O-galloyl- β -D-glucose, 1,3,4,6-tetra-O-galloyl- β -D-glucose, kaempferol, protocatechuic acid, β -amyrin, 24-methylenecycloartenol, β -sitosterol, heptacosane, *n*-nonacosane, shikimic acid, tinyatoxin, choline, camphol, and quercitol derivatives (Koli et al., 2002; Kumar et al., 2010b). Although extensive reports are available on the biological activities and phytochemical constituents of *B. erecta* L. and *E. hirta* L., there is insufficient literature data on the components and bioactivities of their volatile oils.

2. Experimental

2.1. Plant material

Fresh plant materials of *B. erecta* L. and *E. hirta* L. were collected along Arulogun area Ojoo Ibadan, Oyo State, Nigeria, in April 2016. The plants were identified and authenticated at the herbarium unit of Forestry Research Institute of Nigeria (FRIN) Ibadan, by Mr. O. A. Michael. Voucher specimens were deposited with herbarium numbers FHI-110591 and FHI-110590, respectively.

2.2. Isolation of the essential oils

Fresh plant samples of *B. erecta* L. were separated into stems (288.58 g) and roots (86.24 g); and aerial parts of *E. hirta* L. (270.83 g) were chopped, placed in an all glass Clevenger-type apparatus being designed according to British Pharmacopoeia specifications (Zead et al., 2014) and hydrodistilled for 3 h. The essential oil was collected using *n*-hexane and stored in a sealed vial under refrigeration prior to analysis.

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

Essential oils were subjected to GC-MS analysis on an Agilent 7809A gas chromatograph hyphenated with an Agilent mass detector having split/splitless injector interfaced to mass selective detector operating at 70 eV. The other experimental parameters were as follows. The ion source temperature was set at 200 °C over a mass spectral range of *m/z* 50-700 at a scan rate of 1428 amu/sec. GC column was equipped with an HP-5MS column with a length of 30 m, having an internal diameter of 0.25 mm and a film thickness of 0.25 μ m. Oven temperature was programmed as follows: initial temperature 80 °C for 2 min, increased at 10 °C/min to temperature of 240 °C for 6 min. Helium was utilized as the carrier gas at a flow rate of 1 mL/min. Injection volume, linear velocity and pressure were respectively adjusted at 1.0 μ L, 362 cm/s and 56.2 KPa. The oven temperature was set at 60 °C, hold for 1 min to 180 °C for 3 min at 10 °C /min, the final temperature was 280 °C for 2 min at 10 °C /min. Both injector and detector temperatures were fixed at 250 °C. Identification of the essential oil components were based on their retention indices as determined with reference to a homologous series of normal alkane as well as by comparison of their mass spectral fragmentation patterns (NIST data/base/chemstation data system) with the data previously reported in the literature (Adams, 2001).

2.4. Antimicrobial assay

The microorganisms were obtained from the stock cultures of the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria. The test organisms consisted of two Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*); four Gram negative bacteria (*Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*) and four fungi, namely *Candida albicans*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium notatum*. Pour plate and surface plate methods were employed for antibacterial and antifungal activities, respectively (Olaoluwa and Aiyelaagbe, 2015). Oil samples were prepared such that 1 mL of the oil was regarded as 100% concentration; 0.5 mL of this essential oil was taken into 0.5 mL of methanol to give 50% concentration. More

serial dilutions gave different concentrations such as 25%, 12.5%, 6.25% and 3.125%.

2.5. Antioxidant assay

The free radical scavenging activity of the oil samples and standard solutions in methanol was determined based on their ability to react with 2,2'-diphenyl-1-picrylhydrazyl free radical. Accordingly, a methanol solution of DPPH• (0.1 mM) was prepared by dissolving 3.94 mg of DPPH• in 100 mL of methanol. The stock solution of each sample was prepared by dissolving 0.5 mL of each separated oil in 2 mL of methanol to prepare the stock solution. From this solution, 0.5 mL was taken and dispensed into 3 mL of the methanol solution of DPPH•. This gave 100% concentration of each oil sample. Again from the stock solution, 1.0 mL was taken into 1 mL of methanol and 0.5 mL of this solution was dispensed into 3.0 mL of DPPH• methanol solution to give 50% concentration. More serial dilutions were done till 3.125% concentration of each oil extract was achieved. For each of the standards, namely ascorbic acid, butylated hydroxyl anisole (BHA), garlic, ginger and α -tocopherol, 1.0 mg was dissolved in 2 mL of methanol and serial dilutions were done to give concentrations like those of the essential oils. The DPPH solution with oil samples were allowed to incubate

for 10 min and 30 min; and the absorbance of each solution was recorded using a UV Spectrophotometer (Perkin Elmer UV/Visible, Model number: LAMDA 25) at a wavelength of 517 nm. The same procedure was done for the standards. A blank DPPH• solution was analyzed as well and each test was carried out in triplicate. Radical scavenging activity (%) was then calculated using the following formula (Eqn.1):

$$SA(\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (\text{Eqn.1})$$

Where SA(%), A_c and A_s respectively account for the scavenging activity on free radical DPPH in percent, the absorbance of control/blank without extract (at 517 nm) and the absorbance of sample extract (at 517 nm).

3. Results and Discussion

3.1. Chemical composition

Hydrodistillation of *B. erecta* L. stem and root oils and *E. hirta* L. root oil gave colorless oils with 0.60%, 1.08% and 0.56% yields, respectively.

In our characterization, seven compounds were recognized in the volatile oil of *B. erecta* L. stem which accounted for 81.84% of its chemical profile (see Fig. 1 and Table 1). The dominant compound in this oil

Table 1

Composition of volatile oil of *B. erecta* L. stem.

PeakNo	RT(min)	Compounds	MF	TIC (%)
1	20.75	Methyl hexadecanoate	C ₁₉ H ₃₄ O ₂	3.50
2	23.42	1-Octadecene	C ₁₈ H ₃₆	2.67
3	23.61	cis-13-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	2.58
4	23.90	Phytol	C ₂₀ H ₄₀ O	61.31
5	26.03	Squalene	C ₃₀ H ₅₀	6.94
6	27.77	Hexyl hexadecanoate	C ₂₂ H ₄₄ O ₂	4.55
7	27.92	(Z)-9-Octadecenamide	C ₁₈ H ₃₅ NO	0.29
Total				81.84

RT: Retention time in minutes; MF: Molecular formula; TIC(%): Total ion concentration in percentage.

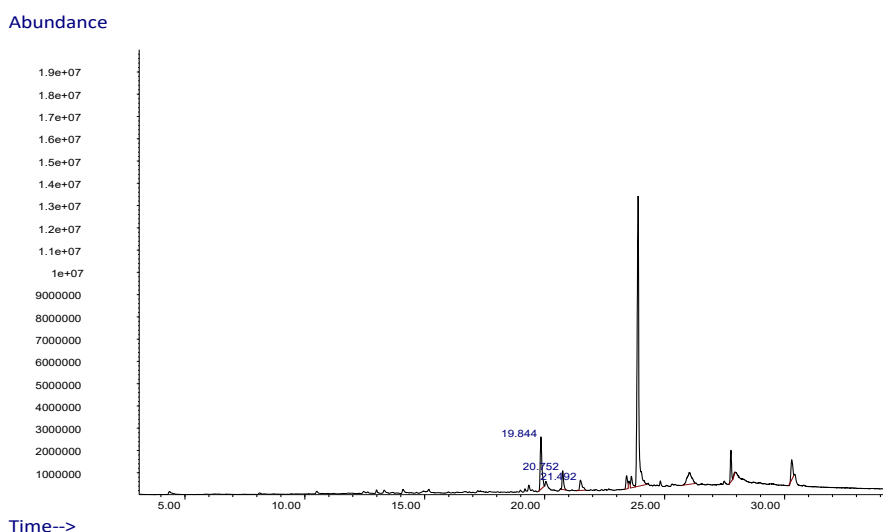
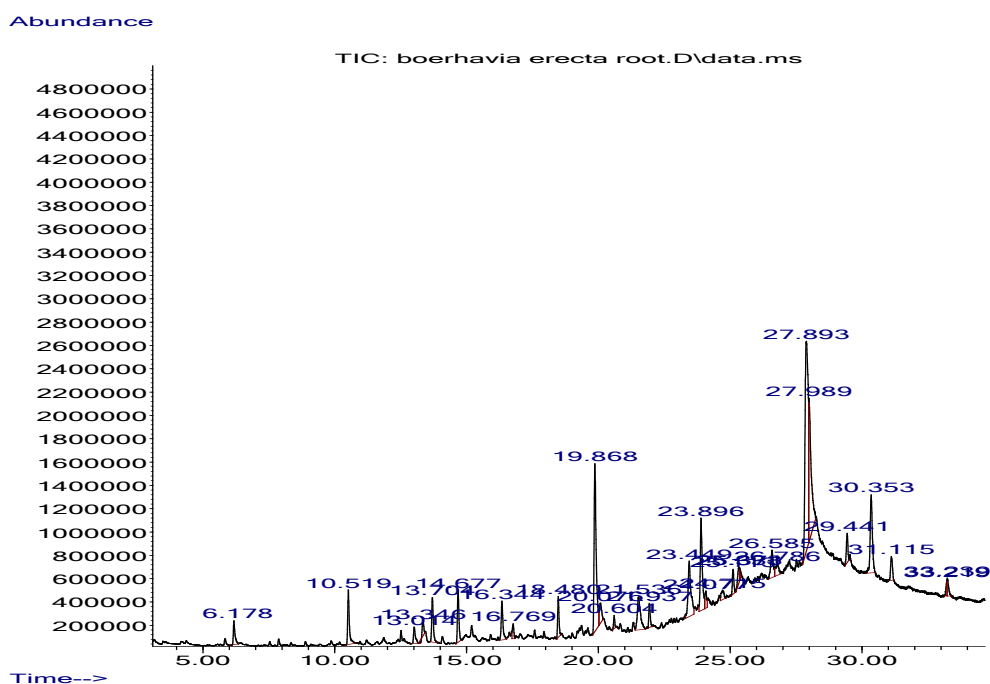


Fig. 1. Chromatogram of volatile oil of *B. erecta* L. stem.

Table 2Composition of volatile oil of *B. erecta* L. root.

Peak No	RT (min)	Compounds	MF	TIC (%)
1	6.18	Dodecane	C ₁₂ H ₂₆	1.34
2	10.52	Tetradecane	C ₁₄ H ₃₀	3.10
3	13.02	Butylated Hydroxytoluene	C ₁₅ H ₂₄ O	1.07
4	13.35	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	0.66
5	13.70	L-calamenene	C ₁₅ H ₂₂	2.53
6	14.68	Hexadecane	C ₁₆ H ₃₄	2.72
7	16.35	Methyl 4-(5-hydroxyl-octyl)benzoate	C ₁₆ H ₂₄ O ₃	2.38
8	16.78	2-Methyloctacosane	C ₂₉ H ₆₀	0.73
9	18.48	Octadecane	C ₁₈ H ₃₈	1.89
10	20.08	Cyclotetradecane	C ₂₀ H ₄₀	1.59
11	20.60	Octacosane	C ₂₈ H ₅₈	0.49
12	23.45	1-Octadecene	C ₁₈ H ₃₈	5.51
13	23.90	Phytol	C ₂₀ H ₄₀ O	6.51
14	24.08	Octadecane	C ₁₈ H ₃₈	1.01
15	24.72	N-[4-bromo-n-butyl]-2-piperidinone	C ₉ H ₁₆ BrNO	0.88
16	25.11	2,6,10,15-tetramethyl heptadecane	C ₂₁ H ₄₄	1.27
17	25.35	Octadecanamide	C ₁₈ H ₃₇ NO	1.31
18	26.59	Cycloeicosane	C ₂₀ H ₄₀	2.29
19	26.79	Oleic acid	C ₁₈ H ₃₄ O ₂	1.32
20	27.94	9-Octadecenamide	C ₁₈ H ₃₅ NO	33.94
21	29.44	Eicosane	C ₂₀ H ₄₂	1.50
22	31.11	Tetracosane	C ₂₄ H ₅₀	1.44
23	33.22	Tricacosane	C ₂₃ H ₄₈	0.54
24	33.24	1-Heptadecene	C ₁₇ H ₃₄	0.91
Total				76.93%

RT: Retention time in minutes; MF: Molecular formula; TIC(%): Total ion concentration in percentage.

**Fig. 2.** Chromatogram of volatile oil of *B. erecta* L. root.

was found to be phytol covering 61.31% of the total composition. Phytol has also been reported in the aerial part of *B. coccinea* as the most abundant constituent (Olaoluwa et al., 2018). The other compounds included squalene (6.94%), hexyl hexadecanoate (4.55%), 1-octadecene (2.67%), *cis*-13-octadecenoic acid, methyl ester (2.58%) and 9-octadecenamide (0.29%). The percentage compositions of groups of natural

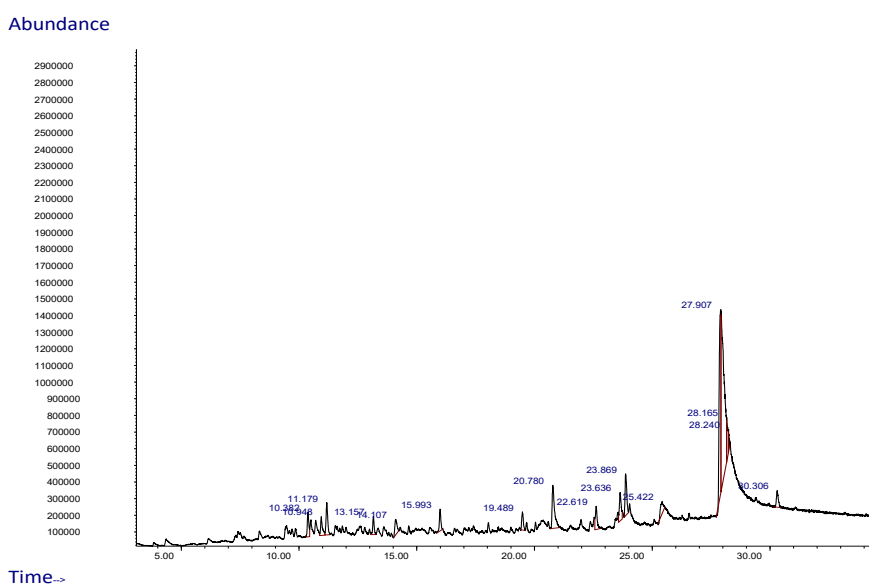
compounds present in the stem oil were alcohol (61.31%), esters (10.63%) and hydrocarbons (8.61%). On the other hand, twenty-four constituents were identified in the volatile oil of *B. erecta* root representing 76.93% of total oil composition (Fig. 2 and Table 2). Root oil was characterized by aliphatic amide (35.25%), hydrocarbon (28.86%), alcohol (6.51%), esters (2.37%) and acid (1.32%). The major component was found to

Table 3

 Composition of the volatile oils of *E. hirta* L. aerial part.

Peak No	RT (min)	Compounds	MF	TIC (%)
1	10.38	β -Elemene	C ₁₅ H ₂₄	2.55
2	10.95	Caryophyllene	C ₁₅ H ₂₄	2.29
3	11.17	<i>p</i> -Menth-3-en-9-ol	C ₁₀ H ₁₈ O	3.64
4	14.11	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	C ₁₅ H ₂₆ O	2.23
5	19.49	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-Hexamethylcyclopenta- γ -2-benzopyran	C ₁₈ H ₂₆ O	1.97
6	20.78	Methyl hexadecanoate	C ₁₇ H ₃₄ O ₂	7.02
7	22.62	Kaur-16-ene	C ₂₀ H ₃₂	2.94
8	23.63	Methyl 9-octadecenoate	C ₁₉ H ₃₆ O ₂	3.53
9	23.87	Phytol	C ₂₀ H ₄₀ O	4.80
10	25.42	Tetradecanamide	C ₁₄ H ₂₉ NO	2.19
11	27.90	(<i>Z</i>)-9-Octadecenamide	C ₁₈ H ₃₅ NO	59.63
Total				92.79

RT: Retention time in minutes; MF: Molecular formula; TIC(%): Total ion concentration in percentage.


Fig. 3. Chromatogram of volatile oil of *B. hirta* L. root.

be 9-octadecenamide (33.94%).

Moreover, eleven compounds were identified in the oil from the aerial parts of *E. hirta* L. representing 92.79% of the oil. The major compound was (*Z*)-9-octadecenamide (60.71%), whereas the other minor components present included methyl hexadecanoate (7.02%), β -elemene (2.54%), phytol (4.80%) and *p*-menth-3-en-9-ol (3.64%) (Fig. 3 and Table 3).

It is noteworthy that phytol was found in the three analyzed oils, while (*Z*)-9-octadecenamide and methyl hexadecanoate were common in the volatile oils of *B. erecta* stem and *E. hirta* L..

3.2. Antioxidant activities

DPPH radical is a common assay used for the fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of its performance (Jin et al., 2017). This assay is known to give reliable information concerning the antioxidant ability of the tested compounds. The principle of DPPH• assay is based upon the color change from purple to yellow as the radical is quenched by the antioxidant agent

(Angeline et al., 2010). The antioxidant activities of the essential oils and the standards were calculated as the percentage inhibition of the samples (see Table 4 and Table 5).

As can be seen in these tables, among the reference antioxidant materials, ascorbic acid displayed the highest scavenging activity followed by butylated hydroxyl anisole, ginger and garlic with α -tocopherol having the least antioxidant potency. Furthermore, the antioxidant activities of *B. erecta* L. stem and root oils were low compared to vitamin C, BHA, ginger and garlic, but were higher than that of α -tocopherol. However, *B. erecta* L. root oil showed better radical free scavenging activity compared to stem oil at 30 min incubation at all concentrations. Root oil gave percentage inhibition of 17-20% at concentrations between 3.125-100% (Table 5). The antioxidant activity of *E. hirta* L., that gave percentage inhibition of 15.7-19.7% at concentrations between 3.125-100%, was higher than that of α -tocopherol. The presence of nitrogen-containing compounds and alcohols in *B. erecta* L. stems oil and *E. hirta* L. aerial parts could be responsible for their mild antioxidant activities (Kumar et al., 2010c) as these

**Table 4**

Free radical scavenging activities on DPPH at 517 nm for 10 min.

Plant Sample	Concentration (%)					
	100	50	25	12.5	6.25	3.125
ES	15.83 ± 0.001	17.99 ± 0.001	18.09 ± 0.001	16.22 ± 0.001	17.99 ± 0.001	17.90 ± 0.001
BER	13.84 ± 0.001	18.15 ± 0.001	16.26 ± 0.001	16.26 ± 0.001	18.33 ± 0.001	16.53 ± 0.001
EH	17.85 ± 0.001	18.81 ± 0.001	17.39 ± 0.001	19.96 ± 0.001	16.15 ± 0.000	15.62 ± 0.001
VIT.C	97.51 ± 0.001	97.63 ± 0.001	97.74 ± 0.001	97.97 ± 0.001	98.19 ± 0.001	97.97 ± 0.000
BHA	96.56 ± 0.000	96.91 ± 0.000	96.91 ± 0.001	96.91 ± 0.000	92.90 ± 0.001	79.15 ± 0.001
α-TCP	14.57 ± 0.001	14.45 ± 0.001	15.61 ± 0.000	16.30 ± 0.001	15.95 ± 0.001	15.84 ± 0.001
GG	66.58 ± 0.001	81.53 ± 0.001	81.16 ± 0.002	67.09 ± 0.001	51.38 ± 0.001	56.28 ± 0.001
GA	26.92 ± 0.002	27.17 ± 0.002	17.42 ± 0.001	15.28 ± 0.002	13.83 ± 0.001	16.23 ± 0.001

DPPH: Scavenging activity of essential oils and standards at 517 nm for 10 min; Conc.(%): Concentration (%); BES: *Boerhavia erecta* L. stem; BER: *Boerhavia erecta* root; EH: *Euphorbia hirta*; α-TCP: α-Tocopherol; GA: Garlic; GG: Ginger; BHA: Butylated hydroxyl anisole; VIT.C: Ascorbic acid.

Table 5

Free radical scavenging activities on DPPH at 517 nm for 30 min.

Plant Sample	Concentration (%)					
	100	50	25	12.5	6.25	3.125
ES	15.95 ± 0.001	17.91 ± 0.001	17.81 ± 0.002	15.66 ± 0.001	17.81 ± 0.001	17.51 ± 0.001
BER	16.77 ± 0.000	18.71 ± 0.000	20.21 ± 0.001	19.86 ± 0.001	17.39 ± 0.000	18.89 ± 0.001
EH	17.39 ± 0.001	18.72 ± 0.002	17.13 ± 0.001	19.70 ± 0.001	17.57 ± 0.001	15.71 ± 0.001
VIT.C	96.81 ± 0.001	96.81 ± 0.000	97.42 ± 0.001	97.91 ± 0.001	98.03 ± 0.001	97.67 ± 0.000
BHA	96.53 ± 0.001	96.99 ± 0.001	96.88 ± 0.000	96.99 ± 0.000	96.88 ± 0.001	87.50 ± 0.001
α-TCP	14.29 ± 0.001	14.06 ± 0.001	15.55 ± 0.001	16.01 ± 0.001	15.55 ± 0.001	17.83 ± 0.001
GG	69.63 ± 0.002	84.50 ± 0.001	87.88 ± 0.001	76.13 ± 0.001	95.75 ± 0.001	63.38 ± 0.001
GA	29.86 ± 0.002	40.02 ± 0.001	23.30 ± 0.002	12.27 ± 0.001	18.96 ± 0.001	19.70 ± 0.001

DPPH scavenging activity of essential oils and standards at 517 nm for 30 min; Conc.(%): Concentration(%); BES: *Boerhavia erecta* L. stem; BER: *Boerhavia erecta* root; EH: *Euphorbia hirta*; α-TCP: α-Tocopherol; GA: Garlic; GG: Ginger; BHA: Butylated hydroxyl anisole; VIT.C: Ascorbic acid.

Table 6Antimicrobial activity of *Euphorbia hirta* L. and *Boerhavia erecta* L. essential oils.

S/N	Samples	Conc. (%)	Diameter of Inhibition Zone (mm)									
			Gram Negative				Gram Positive		Fungi			
			E. c	S. t	P. a	K. p	B. s	S. a	C. a	A. n	P. n	R. s
1	EH	50	12	14	18	16	18	16	14	14	12	
		25	10	14	14	14	14	14	14	12	10	
		12.5	12	12	12	10	12	12	10	10	-	-
		6.25	-	10	10	-	10	10	10	-	-	-
		3.125	-	-	-	-	-	10	-	-	-	-
2	BES	50	20	16	16	16	14	20	18	16	16	18
		25	18	14	14	14	12	16	16	14	14	14
		12.5	14	12	12	12	10	14	14	12	12	12
		6.25	10	10	10	10	10	12	12	10	10	10
		3.125	-	-	-	-	-	10	10	-	-	-
3	BEL	50	16	18	-	14	18	18	16	14	14	12
		25	14	16	-	10	16	16	14	12	12	10
		12.5	12	14	-	-	14	14	10	10	10	-
		6.25	10	12	-	-	12	12	-	-	-	-
		3.125	-	10	-	-	10	10	-	-	-	-
4	Gentamicin (positive)		24	26	24	24	24	26				
5	Tioconazole (positive)								20	22	20	22
6	Methanol (negative)											

EH: *Euphorbia hirta*, BES: *Boerhavia erecta* Stem, BER: *Boerhavia erecta* root; Conc.: Concentration; E.c: *Escherichia coli*; S.t: *Salmonella typhi*; P.a: *Pseudomonas aeruginosa*; K.b: *Klebsiella pneumoniae*; B.s: *Bacillus subtilis*; S.a: *Staphylococcus aureus*; C.a: *Candida albicans*; A.n: *Aspergillus niger*; P.n: *Penicillium notatum*; R.s: *Rhizopus stolonifer*; (-): No activity.

compounds have an ability to donate electron to DPPH radical easily, thereby showing remarkable antioxidant activity (Camila et al., 2013; Costa et al., 2016).

3.3. Antimicrobial activities

In vitro antimicrobial activity of the essential oil of *B. erecta* L. and *E. hirta* L. resulted in a range of growth inhibition patterns against the studied pathogenic microorganisms. The root oil of *B. erecta* L. was moderately active against *S. typhi*, *B. subtilis*, *S. aureus*,

K. pneumoniae and *C. albicans*; and stem oil displayed strong inhibition against *S. aureus*, *E. coli*, *C. albicans* and *R. stolonifer* with zones of inhibition between 14-20 mm and 12-20 mm, respectively, all concentrations ranged between 12.5-50%. The content of polar constituents in stem and root oils may account for the strong inhibition of growth of the microorganism. Polar compounds have been found to have better interaction with microorganisms' cell walls leading to their death (Olaoluwa et al., 2013).

The main constituent in the stem oil, phytol, has been reported to possess antimicrobial, antioxidant and antiparasitic properties (Venkata et al., 2012; Moraes et al., 2014; Pejin et al., 2014). The most abundant component in the root oil, 9-octadecenamide, has revealed anti-inflammatory and analgesic activities (Ghaidaa and Mohammad, 2016). The minor constituents could also contribute to the bioactivity of the oils, as activities observed from essential oils are not merely limited by major constituents (Aboaba and Choudhary, 2015).

The essential oil of *E. hirta* L. showed a moderate inhibition against *S. aureus*, *P. aeruginosa*, *B. subtilis* and *C. albicans* at concentrations between 12.5-50% with inhibition zones ranging from 12-18 mm and 10-16 mm for bacteria and fungus (Table 6) respectively. The oil deterred growth in Gram-positive bacteria more than the Gram-negative bacterial strains.

The most abundant constituent in *E. hirta* L. oil, (Z)-9-octadecenamide, has been stated to exhibit antimicrobial and anti-inflammatory activities (Amutha and Kottai, 2014; Haider et al., 2016).

(Z)-9-Octadecenamide was first isolated from the cerebrospinal fluid of sleep-deprived cats, and has been identified as the signaling molecule that causes sleep. In addition to its sleep-inducing properties, it has other neurological activities like regulation of memory processes, decreasing body temperature and locomotive activity, stimulating Ca²⁺ release, modulation or activation of receptors and effect on perception of pain (Abdelmonim and Saad, 2015). This corroborates its traditional usage as an anxiolytic agent.

Moreover, fatty acid esters identified in the essential oil have been reported to exert antibacterial and antifungal activities and these constituents might have contribution to the antimicrobial properties of *E. hirta* L. (Olaoluwa and Ogunbor, 2015).

4. Concluding remarks

In conclusion, twenty-five, eleven and fourteen compounds were identified in the essential oils from the roots and stems of *B. erecta* L. along with the roots of *E. hirta* L., respectively. The presence of various bioactive compounds in these chemical profiles proved that both plants are of pharmaceutical importance. In the present report, the essential oils from the studied plants displayed mild antioxidant and average

antimicrobial properties that may further corroborate their ethnomedicinal uses.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

Authors acknowledge the use of J-laboratory facilities, Department of Chemistry, University of Ibadan, in essential oil isolation and are greatly thankful to Mr. Festus, of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria, for his assistance on antimicrobial assay.

References

- Abdelmonim, O.A.H., Saad, M.H., 2015. Chemical composition and antimicrobial activity of Sudanese *Lupinus termis* L. root extracts. *J. Pharm. Innov.* 4(5), 1-4.
- Aboaba, S.A., Choudhary, M.I., 2015. Chemical composition and biological activities of the volatile oils of *Palisota hirsuta* (Thunb) K. Schum and *Trema orientalis* (L) Blume. *Int. J. Chem.* 7(2), 21-26.
- Adams, R.P., 2001. Identification of Essential Oil Composition by Gas Chromatography/ Quadrapole. Allured Publishing, Carol Stream, IL.
- Adedapo, A.A., Abatan, M.O., Akinloye, A.K., Idowu, S.O., Olorunsogo, O.O., 2003. Morphometric and histopathological studies on the effects of some chromatographic fractions of *Phyllanthus amarus* and *Euphorbia hirta* on the male reproductive organs of rats. *J. Vet. Sci.* 4(2), 181-185.
- Adedapo, A.A., Shabi, O.O., Adedokun, O.A., 2005. Anthelmintic efficacy of the aqueous extract of *Euphorbia hirta* (Linn.) in Nigerian dogs. *Arch. Vet. Sci.* 75(1), 39-47.
- Amutha, I.D.J., Kottai, M.A., 2014. Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Saccharum spontaneumlinn*. *Int. J. Pharm. Pharm. Sci.* 6(2), 755-759.
- Angeline, T., Sreenivasan, S., Lachimanan Y. L., Sivaramakrishnan S., Surash, R., 2010. Antioxidant activity and total phenolic content of methanol extracts of *Ixora coccinea*. *Pharm. Biol.* 48(10), 1119-1123.
- Aworinde, D., Nwoye, D., Jayeola, A., Olagoke, A., Ogundele, A., 2009. Taxonomic significance of foliar epidermis in some members of *Euphorbiaceae* family in Nigeria. *J. Bot.* 4(1), 17-28.
- Calderón-Montaño, J.M., Burgos-Morón, E., Pérez-Guerrero, C., López-Lázaro, M., 2011. A review on the dietary flavonoid kaempferol. *Mini-Rev. Med. Chem.* 11(4), 298-344.
- Camila, C.M.P.S., Mirian, S.S., Vanine, G.M., Luciana, M.C., Antonia, A.C.A., Guilherme, A.L.O., Jéssica, P.C., Damião, P.S., Rivelilson, M.F., Reinaldo, N.A., 2013. Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. *J. Neurosci.* 2013, 1-9.
- Camilo, C.J., Alves Nonato, C.d.F., Galvao-Rodrigues,



- F.F., Cosa, W.D., Clemente, G.G., Sobreira Macedo. M.A.C., da Costa, J.G.M., 2017. Acaricidal activity of essential oils: a review. *Trends Phytochem. Res.* 1(4), 183-198.
- Costa, J.P., Islam, M.T., Santos, P.S., Ferreira, P.B., Oliveira, G.L., Alencar, M.V., Paz, M.F., Ferreira, É.L., Feitosa, C.M., Citó, A.M., Sousa, D.P., Melo-Cavalcante, A.A., 2016. Evaluation of antioxidant activity of Phytol using non- and pre-clinical models. *Curr. Pharm. Biotechnol.* 17(14), 1278-1284.
- Ghaidaa, J.M., Mohammad, J.I.H., 2016. Anti-bacterial, antifungal activity and chemical analysis of *Punica grantanum* (Pomegranate peel) using GC-MS and FTIR spectroscopy. *Int. J. Pharmacog. Phytochem. Res.* 8(3), 480-494.
- Haider, M.H., Imad, H.H., Ibraheem, O.A., 2016. Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of *Adiantum capillus-veneris* using GC-MS and FT-IR spectroscopy. *Int. J. Pharmacog. Phytochem. Res.* 8(3), 369-385.
- Jin, H.P., Yun, J.L., Yeon, H.K., Ki, S.Y., 2017. Antioxidant and antimicrobial activities of Quinoa (*Chenopodium quinoa* Willd.) seeds cultivated in Korea. *Prev. Nutr. Food Sci.* 22(3), 195-202.
- Koli, M.C., Choudhary, R., Kumar, S., Thakur, S., Shukla, D., Saxena, R.C., 2002. An isoflavone glycoside from the stem of *Euphorbia hirta* Linn as antimalarial compound. *Asian J. Chem.* 14(3-4), 1673-1677.
- Kumar, P.P., Kumaravel, S. Lalitha, C., 2010a. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *Afr. J. Biochem. Res.* 4(7), 191-195.
- Kumar, S., Malhotra, R., Kumar, D., 2010b. *Euphorbia hirta* its chemistry, traditional and medicinal uses and pharmacological activities. *Pharmacogn. Rev.* 4(7), 58-61.
- Kumar, S., Malhotra, R., Kumar, D., 2010c. Antidiabetic and free radicals scavenging potential of *Euphorbia hirta* flower extract. *Indian J. Pharm. Sci.* 72(4), 533-537.
- Mohammedhosseini, M., Mahdavi, B., Akhlaghi, H., 2013. Characterization and chemical composition of the volatile oils from aerial parts of *Eryngium bungei* Bioss. (Apiaceae) by using traditional hydrodistillation, microwave assisted hydrodistillation and head space solid phase microextraction methods prior to GC and GC/MS analyses: a comparative approach. *J. Essent. Oil Bear. Plants* 16(5), 613-623.
- Mohammedhosseini, M., Sarker, S.D., Akbarzadeh, A., 2017. Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities. A review. *J. Ethnopharmacol.* 199, 257-315.
- Moraes, J., de Oliveira, R.N., Costa, J.P., Junior, A.L.G., de Sousa, D.P., Freitas, R.M., 2014. Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease *Schistosomiasis mansoni*. *PLoS Negl. Trop. Dis.* 8(1), e2617.
- Nunes, H.S., Miguel, M.G., 2017. *Rosa damascene* essential oils: a brief review about chemical composition and biological properties. *Trends Phytochem. Res.* 1(3), 111-128.
- Nugraha, A.S., 2009. Bioactive compounds from *Boerhavia erecta* Linnaeus: an African medicinal plant, Research thesis, School of Chemistry, University of Wollongong, retrieved online on 31 May, 2017.
- Nugraha, A.S., Hilou, A., Vandegraaff, N., Rhodes, D.I., Haritakun, R., Keller, P.A., 2015. Bioactive glycosides from the African medicinal plant *Boerhavia erecta* L. *Nat. Prod. Res.* 29(20), 1954-1958.
- Olaoluwa, O.O., Aiyelaagbe, O.O., 2015. Phytochemical investigation and antimicrobial screening of *Cardiospermum grandiflorum* (Sweet) [Sapindaceae]. *Int. J. Pharma Sci. Res.* 6(2), 348-351.
- Olaoluwa, O.O., Aiyelaagbe, O.O., Irwin, D., Reid, M., 2013. Novel anthraquinone derivatives from the aerial parts of *Antigonon leptopus* Hook & Arn. *Tetrahedron* 69(33), 6906-6910.
- Olaoluwa, O.O., James, D.A., Adigun, O.A., 2018. Volatile oil analysis of aerial parts of *Boerhavia coccinea* (Mill.). *Nat. Prod. Res.* 32(8), 959-962.
- Olaoluwa, O.O., Ogunbor, F., 2015. Phytochemical screening, antimicrobial properties and essential oil constituents of *Combretum sordidum* exell. *Int. J. Pharm. Sci. Res.* 6(3), 1176-1180.
- Patil, K.S., Bhalsing, R.S., 2016. Ethnomedicinal uses, phytochemistry and pharmacological properties of genus *Boerhavia*. *J. Ethnopharmacol.* 182, 200-220.
- Pejin, B., Savic, A., Sokovic, M., Glamoclija, J., Ciric, A., Nikolic, M., Radotic, K., Mojovic, M., 2014. Further *in vitro* evaluation of antiradical and antimicrobial activities of phytol. *Nat. Prod. Res.* 28(6), 372-376.
- Rao, K., Karthik, L., Humalai, E., Srinivasn, K., Kumar, G., 2010. Antibacterial and antifungal activity of *Euphorbia hirta* L. leaves - a comparative study. *J. Pharm. Res.* 3(3), 548-549.
- Shareef, M.I., Gupta Ayushi, S.M., Gupta, S., 2017. Antioxidant and anticancer study of *Boerhavia erecta*. *Int. J. Curr. Microbiol. Appl. Sci.* 6(9), 879-885.
- Sharma, P., Mohan, L., Srivastava, C.N., 2009. *Amaranthus oleracea* and *Euphorbia hirta*: natural potential larvicidal agents against the urban Indian malaria vector, *Anopheles stephensi* Liston Culicidae. *Parasitol. Res.* 106(1), 171-176.
- Stintzing, F.C., Kammerer, D., Schieber, A., Adama, H., Nacoulma, O.G., Carle, R., 2004. Betacyanins and phenolic compounds from *Amaranthus spinosus* L. and *Boerhavia erecta* L. *Z. Naturforsch.*, 59c, 1-8.
- Suriyavathana, M., Paramesiwari, G., Penislus, S.S., 2012. Biochemical and antimicrobial study of *Boerhavia erecta* and *Chromolaena odorata* (L.) King & Robinson. *Int. J. Pharm. Sci. Res.* 3(2), 465-468.
- Venkata, R.B., Samuel, L., Pardha, S.M., Narashimha, R.B., Naga, V.K.A., Sudhakar, M., Radhakrishnan, T., 2012. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian J. Pharm. Clin. Res.* 5(2), 99-106.
- Zead, H., Khaldun, M., Yuliya, T., Refat, A.G., Hassan, S., Hassan, A., 2014. Analysis of essential oil in Jerusalem artichoke (*Helianthus tuberosus* L.) leaves and tubers by gas chromatography-mass spectrometry. *Adv. Pharm. Bull.* 4(2), 521-526.