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Different methods for extraction of some chemical constituents from different organs of *Ipomea carnea* and their antioxidant activity

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The study aim was to extract different volatile, lipid constituents and flavonoids from different parts of *Ipomea carnea* (*IC*), in addition to evaluate their antioxidant activity. The volatile constituents of different organs (flowers, leaves and stems) of *Ipomea carnea* (*IC*) were extracted using different methods (hydrodistillation (HD), microwave (MAE), solvent extraction (SE) and solid-phase-microextraction (SPME) and all of them were analyzed using GC/MS. The total phenolic compounds (TPC) were assayed in different extracts using the Folin-Ciocalteau's phenol protocol with minor modification and total flavonoidal content (TFC) was determined using the colorimetric method with the UV-VIS spectrophotometer. The different percentages of constituents detected in the essential oils (EOs) of the studied four different methods for flowers, leaves and stems were as follows: Microwave (MAE) were (98.1%), (97.8 %), (96.2 %), and in hydrodistiation (HD) were (95.5 %), (95.4 %), (93.8 %), in solvent extraction were (96.66%), (95%), (92.19%) and in SPME were (94.9 %), (94.45 %), (92.18%) respectively. The lipid constituents from flowers, leaves and stems were extracted with pet. ether and fractionated to fatty alcohols, hydrocarbons, unsaponifiable matters and fatty acid methyl esters which were identified by GLC and /or GC/MS. The phenolic constituents were isolated from the ethyl acetate fraction of the methanolic extract of the flowers led to identification of umbliferon, kaempferol and kaempferol -3-*O*-glucoside. The study of the antioxidant activity of different extracts was studied. The microwave extraction method is the best method for extraction of essential oil constituents, the flavonoidal constituents were isolated from the flowers and the leaves alcoholic extract exhibited the highest antioxidant activity using DPPH assay (IC_{50} =1.11 mg/ml)

Keywords: ***Ipomoea carnea,*** **Convolvulaceae family, extraction methods, lipid constituents, flavonoids and antioxidant activity.**

INTRODUCTION

The *IC* (Convolvulaceae family) commonly growing in Egypt, it is an ornamental tree and is a class of medicinally important plant for its antimicrobial, anticancer, anti-inflammatory, wound healing, skin infections as a topical antiseptic, anti- rheumatic remedy and antioxidant activities for many other medicinal activities.

Preliminary phytochemical screening of *IC* proved the presence of phenolic compounds, terpenoids, flavonoids and steroids (Shaltout et al. 2006; Huang et al., 2008; Elija et al. 2010; Hassan et al. 2015). The GC/MS of the hexane extract of *IC* reported the presence thirteen compounds in which hexadecanoic acid, stearic acid, 1,2-diethyl phthalate, n-octadecanol, octacosane, hexatriacontane, tetraacontane and 3-diethylamino-1-propanol are the most relevant (Haraguchi et al. 2003; Nusrat et al. 2014). It was found that, the aqueous alcoholic extract of *IC* contains a considerable amount of flavonoids and phenols. The flowers contain maximum amount of phenolic compounds while stems contain their minimum amount (Phenolic values between 45 to 73 mg catechol equivalent/gm) (Deepa and Shukla, 2015). Sahayaraj and Ravi in 2008 identified the secondary metabolites isolated from the n-hexane fraction of the alcoholic extract of *IC* as dodecyl-p-coumarate, methyl-p-coumarate, octyl-p-coumarate, umbelliferon, scopoletin, 3-oleanone, β-sitosterol and stigmasterol. Haraguchi et al., in 2003 and Hueza et al., in 2005 have been identified the toxic alkaloids of *IC* as swainsonine, calystegines B_1, B_2, C_1 and B_3. Ambiga et al., 2007 have been found that the fresh flowers of *IC* to contain kaempferol and it's 3-*O*-β-D glucoside which were displayed a strong wound healing activity, in addition to 2-C- methyl-D-erythritol and quercetin.

Forty-two components were identified, representing 97.1% of the total hydro-distilled essential oil from flowering aerial parts of *I. obscura* using gas GC-FID and (GC/MS). The major constituents were α-bulnesene (23.8%), α-humulene (13.7%) and seychellene (11.2%), while the other minor constituents were α-guaiene (8.3%), β-caryophyllene (7.1%), γ-terpinene (4.2%), palmitic acid (3.0%) and β-elemene (2.7%), and the oil is rich in sesquiterpene hydrocarbons (78.4%) (Joshi, 2015).

After surveying the available literature it was found that there is no data reported on volatile and lipid constituents of the investigated plant. So, target of the present study is the extraction of different volatile, lipid constituents and flavonoids from different parts of *IC,* in addition to evaluate their antioxidant activity

MATERIALS AND METHODS

Plant Material

The aerial parts of *IC* was collected from Zagazig-Sharkia governorate, in May 2016 and identified by Dr. Mohammed Elgebaly at Medicinal and Aromatic Plants Researches Department, National Research Center (NRC). Voucher specimen was deposited at the herbarium of the NRC. (Herbarium specimen number: 12702).
Immediately after collection, the flowers, stems and leaves were separated and air-dried for two weeks under laboratory conditions at 28±2 °C. The dried materials were partially ground using a domestic blender and transferred into three plastic jars of 1 L each, and stored at room temperature for further studies. Extraction of essential oils
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Hydrodistillation (HD)

The essential oils were obtained by steam distillation from the air-dried flowers, stems and leaves samples (100 g each) of *IC* using a Clevenger apparatus for 4 h. The volatile distillate was collected, then the distillate was taken with diethyl ether, dried over anhydrous sodium sulfate to remove the moisture and the ether was distilled off over a water bath maintained at 40°C. The remainder oils were weighed and stored at 5°C for further analysis.

Microwave Assisted Extraction (MAE)

The essential oil was obtained from different organs (flowers, stems and leaves) (100 g each) of the plant by hydrodistillation for 60 min using a Clevenger-type apparatus placed in a modified microwave oven (MARS 240v/ 50 Hz). During distillation, time, temperature, pressure and power were monitored and controlled with the “easy-control” software package of the system. Microwave power applied to the plant material was controlled by a shielded thermocouple inserted directly into the flask. The oven was operated for 55 min at 800 Watts up to 90ºC, then followed by 5 min of ventilation. The essential oils obtained at different conditions were collected in amber colored vials, dehydrated with anhydrous sodium sulphate, capped under nitrogen, and kept at 4 ºC until being analyzed (Kosar et al. 2007; Árpád et al. 2011).

Solvent extraction (SE)

About 100 g of different organs (flowers, stems and leaves) of *IC* were extracted by maceration with hexane for 24 hrs. (3x1L). Each combined extract was evaporated *in vacuo* at 40˚ C till free from solvent. The combined solvents were dried over anhydrous sodium sulfate and

evaporated then the defatted extract was collected.

Solid phase micro extraction (SPME)

SPME is becoming widely used as an extraction method. It affords a number of advantages in simplifying sample preparation, increasing reliability, selectivity, sensitivity and reducing the cost and time of analysis. The majority of early application for the extraction of volatile components from plants is most widely used. The versatility of fiber SPME is enhanced by the possibility of direct insertion into the sample matrix for the analysis of less volatile components, direct insertion methods, often require sample agitation and require longer extraction times than other methods.

Polydimethylsiloxane fibres (100 µm) were mounted in a SPME manual holder (Supelco, Bellefonte, PA, USA). Fibers were conditioned prior to analyses, according to the manufacturer recommendations. The fiber was maintained over the sample for 30 min. After the sampling time, the fiber was withdrawn into the needle, then transferred immediately to the injection port of GC/MS.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

The obtained residues of hexane extracts of different parts using different extraction methods were subjected to the GC-Ms analysis. The essential oil samples were carried out using gas chromatography-mass spectrometry instrument stands at the Laboratory of medicinal and aromatic plants, National Research Center, Egypt with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 mL/min at a split ratio of 1:10 and the following temperature program: 80 ºC for 1 min; rising at 4 ºC/min to 300 ºC and held for 1min. The injector and detector were held at 220 and 200 ºC, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library). The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology (NIST) published as well as those published by Adams.

Extraction of lipid constituents

About 5 g of the oily residue obtained from flowers, leaves and stems *IC* by solvent extraction method (with hexane) were subjected to saponification process to afford the unsaponifiable materials, fatty acid methyl esters and acetone insol. fraction.

2.5. GC/MS of unsaponifiable matters, acetone insoluble and fatty acid methyl esters (FAMEs)

The GC-MS analysis for unsaponifiable matters and acetone insoluble were carried out using the following specifications, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d., 0.25 *µ*m film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 50^{o} C for 3 min; rising at 5^{o} C/min to 300^{o} C and held for 20 min. The injector and detector were held at 280^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 *µ*L of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. While the conditions for FAMEs are: Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d., 0.25 *µ*m film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 80 ^{o}C for 1 min; rising at 4.0 ^{o}C/min to 300 ^{o}C and held for 1 min. The injector and detector were held at 240 ^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 *µ*L of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 40-450.

Extraction and isolation of flavonoidal constituents

About 500 g of dried powdered flowers of *IC* were extracted with hexane in a Soxhlet for 24 hrs., followed by maceration with methanol (80%,

3x2.5L). The hydro-alcoholic extract was evaporated *in vacuo* at 50° C till free from methanol and diluted with hot distilled water (600 ml). The aqueous filtrate was partitioned with ethyl acetate (400 ml x 3), the combined solvents were dried over anhydrous sodium sulfate and evaporated till dryness.

The ethyl acetate fraction (3 g) was applied onto silica gel column firstly eluted with chloroform: ethyl acetate (50:50) then increasing the polarity with ethyl acetate and methanol respectively, to obtain nine fractions each three of them were pooled together to give three main fractions (A-C). All the fractions were rechromatographed over a small Sephadex LH-20 column eluted with methanol to afford three compounds (I, II, III) in a pure form.

Determination of Total Phenolic Content (TPC)

Dry extract from hydroalcoholic extracts of flowers, leaves and stems of *IC* were pulverized and homogenized in a mortar with 1 mL of methanol to facilitate the extraction, the extracts were centrifuged at 10.000 rpm for 10 minutes at room temperature to collect the supernatant (methanol extract) to be used for the determination of secondary metabolites. The TPC were assayed in different extracts using the Folin-Ciocalteau's phenol protocol with minor modification. 500 µL of Folin Ciocalteau's reagent and 0.45 mL of sodium carbonate (7.5% w/v) were added to 1 mL of total volume sample. After the incubation at room temperature for 2 h, the absorbance at 765 nm of the samples was detected in UV-VIS spectrophotometer and referred to a standard curve for chlorogenic acid prepared in the range of 0–50 mg mL^{-1}. All determinations were performed in triplicate (Singleton VL and Rossi, 1965).

Determination of Total Flavonoidal Content (TFC)

The TFC was determined using the colorimetric method of Kim *et al.* (2003). Methanol extract of different samples was added to the solution of 5% (w/v) sodium nitrite ($NaNO_2$) and incubated for 5 minutes with the 10%(w/v) of aluminium chloride ($AlCl_3$) solution; after 5 minutes, 0.5 mL of 1 M sodium hydroxide (NaOH) were added. The absorbance of the samples was detected after 15 minutes at 510 nm with the UV-VIS spectrophotometer, and referred to a calibration curve done with rutin (1mg mL^{-1}) as standard. Each analysis was repeated three times.

Antioxidant Activity using DPPH Radical-Scavenging Method

The antioxidant activity of hydroalcoholic extracts of flowers, leaves and stems of *IC* was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical DPPH according to (Viuda-martos *et al*., 2010), with some modifications. A volume of 50 µL of a methanolic stock solution of flowers, leaves and stems at three different concentrations was put into a cuvette, and 2 mL of 6 X 10^{-5} mol L^{-1} methanolic solution of DPPH was added. The mixtures were well shaken in a vortex (2500 rpm) for 1 min and then placed in a dark room. The decrease in absorbance at 517 nm was determined with a UV-VIS spectrophotometer after 30 min for all samples. Methanol was used to zero the spectrophotometer. Absorbance of the radical without sample was used as control.

RESULTS

. **Composition of the essential oils (EOs)**

The EOs obtained from the flowers, leaves and stems of *IC*, were extracted by the conventional HD and MAE, SE and SPME, then analyzed by GC/MS. The EOs of *IC* showed distinct unpleasant odors, this might be due to the presence of caryophyllene, geraniol, linalool, 1,8-cineole *α*-pinene, myrcene, ocimene, terpinen-4-ol, *α*-terpineol, and sesquiterpenes (Eyres et al. 2007). The results in Table 5 showed that, the total oil extraction yield of flowers was highest by using MAE followed by SE and the lowest yield was obtained by SPME of all organs. The major oil components extracted by the four methods are oxygenated sesquiterpenes (76.4% and 70.08%) within MAE and HD of flowers and hydrocarbons were found as major in SE of stems (45.87%) and in SPME of flowers (48.78%). The results revealed that, MAE gave the highest yield of oil as well as shown to enhance the extraction efficiency of oxygenated sesquiterpenes.

Tables (1- 4) revealed the presence of 46,52,64 and 86 compounds in flowers, leaves and stems of *IC* EOs using four techniques (MAE, HD, SE and SPME) respectively.

In flowers using SE (Table 3), the obtained EOs were found to contain other groups of compounds which mainly were hydrocabons and esters like hexatriacontane, methyl palmitate and nonacosane (12.17%, 9.32% and 9.18%,

respectively). While using SPME (Table 5), the EOs of flowers were rich in hydrocarbons compounds which were present in larger amounts (48.78%) than in the SE (35.42%).

Table 1: GC/MS of EOs using MAE extraction of flowers, leaves and stems of *IC.*

Peak No.	R_t (min.)	%			Mass data			Compounds
		flowers	Leaves	stems	M^+	B.P	Fragments	
1	3.98	-	0.52	3.5	128	57	99(10),85(42),71(28)	n-Nonane
2	4.07	-	0.2	-	106	91	77(25), 55(12), 105(30)	1,3 -Dimethylbenzene
3	6.31	-	0.27	3.75	136	93	79(28), 69(85), 55 (15)	α-Myrecene
4	6.62	-	-	5.42	136	93	119(10),71(32),65(8)	α-Phellandrene
5	6.9	-	0.51	-	136	93	77(41), 105(11), 65(12)	α-Thujene
6	7.22	-	0.20	-	136	93	121(95), 105(28), 77(45)	2-Carene
7	7,32	-	-	1.68	136	121	121(18),91(52),77(41)	α-Terpinene
8	7.56	-	1.73	6.39	134	119	134(29),91(32), 77(14)	O-Cymene
9	7.64	-	3.70	30.34	136	68	107(22),79(42), 53(21)	Limonene
10	8.66	-	2.44	13.70	136	93	121(35), 105(19), 77(35)	ɣ-Terpinene
11	10.37	-	0.23	-	154	93	107(28), 71(92), 55(91)	Linalool
12	18.41	-	0.29	-	194	138	123(15), 96(45), 82(45)	Theaspirine A
13	19.13	-	0.25	-	194	138	109(22), 96(43), 82(38)	Theaspirine B
14	19.68	-	5.31	-	204	121	161(31), 136(50),93(91)	Elemene
15	22.21	-	0.59	-	204	81	147(40), 107(71), 53(42)	Germacrene A
16	23.41	7.16	13.83	4.54	204	93	133(75),105(56), 69(88)	Trans-caryophyllene
17	23.85	-	0.22	-	204	93	161(67), 121(87),105(82)	(+)Aromadendrene
18	23.96	0.09	0.61	0.96	204	93	119(92),107(35),69(48)	Trans-Bergamotene
19	24.66	-	0.16	1.86	204	44	147(28),105(52),81(59)	(-)α-Selinene
20	24.94	0.08	3.33	-	204	93	147(19),121(25),80(35)	α -Humulene
21	26.00	8.50	47.16	6.37	204	161	119(52),105(87),91(82)	Germacrene-D
22	26.31	-	-	0.96	252	43	153(15), 119(18), 71(22)	t-Butyl oxy formamide
23	26.57	-	3.75	-	204	121	161(22),107(52),93(89)	Bicyclogermacrene
24	27.46	-	-	1.16	280	57	191(10), 81(22), 69(35)	Tetraneurin-A
25	27.51	-	1.52	-	204	161	134(62),105(81),91(65)	Cadinene(S)
26	27.76	-	-	1.42	240	43	169(8), 71(48), 57(62)	2,6,10-Trimethyl tetradecane
27	27.86	-	2.06	-	204	121	161(28),136(43),93(91)	δ-Elemene
28	29.09	-	6.98	-	204	121	189(19),133(35),67(71)	δ-Gurjunene
29	29.40	0.31	-	-	220	43	119(10), 91(38), 59(32)	E- Farnesene epoxide
30	29.93	-	0.18	-	222	81	207(10),123(22), 43(35)	1,6-Germacrdien -5-ol
31	30.10	73.32	0.40	-	220	79	121(28),109(58),93(92)	Caryophyllene oxide
32	30.67	0.21	-	-	206	43	163(9), 79(42), 55(42)	Patchulane
33	31.14	0.43	-	-	220	43	176(18), 121(33), 91(59)	α-Bisabolene epioxide
34	31.77	1.54	-	-	220	43	205(30), 119(62), 91(79)	(-) Spathulenol
35	32.62	-	0.21	-	222	95	204(25),161(45),121(56)	t-Cadinol
36	32.85	1.67	-	-	220	43	138(12), 96(42), 67(85)	4-Hexadecen-6-yne
37	33.06	-	0.68	-	222	95	204(31),121(75),71(45)	t-Muurolol
38	33.89	0.34	-	-	196	43	136(21), 79(35), 56(38)	Geranyl acetate
39	33.92	0.06	-	1.86	278	149	157(10),98(25),57(65)	Dibutylphthalate
40	33.97	-	0.3	-	268	71	155(11),99(42),85(75)	2-Methyloctadecane
41	34.30	0.15	-	-	220	43	105(24), 79(32), 67(62)	Longipinene epoxide
42	34.79	0.65	-	-	220	43	131(52),105(85),93(78)	Ledene oxide (II)
43	41.18	0.81	-	-	268	43	110(28),71(45),58(87)	6,10,14 Trimethyl-2-penta decanone.
44	41.24	2.78	0.17	1.87	310	57	127(18),99(42),85(92)	Docosane
45	54.47	-	-	4.58	314	43	218(12), 98(22),73(56)	1,1-Dimethoxy octadecane
46	56.88	-	-	5.84	354	43	201(21),129(49),73(89)	Propyl2,3dioxy-9-octdecenoate

Table 2: GC/MS of EOs using hydrodistllation of flowers, leaves and stems of *IC.*

Peak No.	R_t (min.)	%			Mass data			Compounds
		flowers	leaves	stems	Mol. Wt.	b.p.	Fragments	
1	6.31	-	-	0.60	136	93	121(8),69(72),43(85)	α-Myrecene
2	6.90	-	-	1.36	136	93	105(15),91(55),77(32)	1-Phellandrene
3	7.55	-	-	2.01	134	119	103(10),91(31),65(12)	*O*-Cymene
4	7.63	-	-	9.06	136	68	107(25),93(68),79(35)	Limonene
5	7.75	-	-	1.03	154	93	108(34),81(55),43(80)	1,8 Cineole
6	8.65	-	-	4.22	136	93	121(18),91(52),77(41)	α-Terpinene
7	10.34	-	0.64	-	154	71	121(32),93(97),55(67)	Linalool
8	12.08	0.12	-	-	142	42	98(8),70(15), 57(33)	n- Nonanal
9	18.36	-	2.39	4.01	194	138	123(15),109(22),96(42)	Theaspirane-B
10	19.81	0.10	1.35	2.86	204	121	161(31),136(56),93(89)	e-Elemene
11	21.52	-	0.45	-	204	119	161(72),91(52),55(22)	α-Copaene
12	21.96	-	1.23	0.67	190	69	121(55),105(31),91(18)	α-Damascenone
13	23.36	13.12	14.48	7.86	204	93	189(15),133(69),105(62)	Trans-caryophyllene
14	23.62	0.19	-	-	204	79	123(21), 81(60), 43(85)	α-Bourbonene
15	23.82	-	0.36	-	204	161	147(15),121(52),105(71)	Muurolene
16	23.92	0.09	2.70	1.75	204	93	119(92),107(32),69(54)	Trans-α-bergamotene
17	24.89	-	2.9	1.92	204	93	147(22),107(15),80(35)	α-Humulene
18	25.63	0.35	-	-	204	43	161(15),119(31),69(42)	α-Farnesene
19	25.95	7.66	17.73	7.96	204	161	119(52),105(88),91(78)	Germacrene-D
20	26.10	-	0.34	-	192	177	161(22),105(31),79(32)	α-Ionone
21	26.52	-	0.74	-	204	121	161(25),107(58),93(85)	Germacrene-B
22	26.70	-	-	1.18	260	57	161(21),85(48),71(65)	1-Chloro hexadecane
23	26.71	-	1.03	-	232	57	204(10),105(25),71(78)	1-Chloro-tetradecane
24	27.03	-	0.55	-	204	67	189(32),107(71),93(89)	Germacrene-A
25	27.46	0.14	0.67	0.84	204	161	134(69),105(82),91(59)	α-Cadinene
26	28.81	66.3	23.21	17.93	220	79	133(18),106(85),91(82)	Caryophyllene oxide
27	29.40	0.13	-	-	220	43	119(10), 91(38), 59(32)	E- Farnesene epoxide
28	29.51		0.63	-	220	96	177(18),123(60),81(65)	Aromadendrene oxide
29	29.93	1.14	2.97	3.66	220	43	205(30),119(62),91(79)	(-) Spathulenol
30	30.60	0.21	-	-	196	43	106(6), 79(28), 56(54)	Linalyl acetate
31	31.18	-	3.18	2.24	220	96	138(68),109(92),55(52)	Humulene oxide
32	31.99	-	5.08	9.75	220	119	187(18),105(69),91(87)	Iso spathulenol
33	32.15	0.10	-	-	152	43	123(38), 81(48), 55(32)	2,2-Dimethyl oct-3,4-dienal
34	32.68	-	1.09	0.75	220	43	205(18),177(35),82(67)	Longipinocarveol (trans)
35	32.99	-	1.14	2.52	222	95	204(22),161(53),81(48)	α-Cadinol
36	33.14	0.93	0.54	0.66	220	133	176(25),105(28),91(52)	Khusinol
37	33.61	0.43	0.51	0.94	220	81	176(18),121(33),91(59)	α-Bisabolene epioxide
38	34.06	0.41	-	-	222	43	136(25), 79(28), 56(61)	Farnesol
39	34.30	0.89	-	-	220	43	105(24), 79(32), 67(62)	Longipinene epoxide
40	34.35	-	-	1.75	220	159	177(24),105(51),91(63)	Neoclovenoxide alcohol
41	34.37	0.99	0.58	-	220	159	131(52),105(85),93(78)	Ledene oxide (II)
42	38.86	0.95	-	-	262	43	91(10), 81(18), 55(28)	4,6,9-Nonadecatriene
43	39.53	0.40	1.09	1.41	268	43	110(28),71(45),58(87)	6,10,14 -Trimethyl-2-penta decanone
44	40.32	-	-	4.86	278	149	223(15),167(12),57(28)	Iso butyl phthalate
45	46.56	-	0.75	-	282	55	253(9),85(48),71(75)	3-Methyl nonadecane
46	47.72	-	1.82	-	282	57	141(8),85(45),71(75)	Eicosane
47	48.14	-	0.32	-	296	71	152(8),81(32),57(63)	Phytol
48	48.50	0.21	-	-	296	43	99(8), 71(25), 57(48)	2-Methyl-eicosane
49	49.30	0.64	-	-	296	57	85(44), 71(69), 43(95)	n-Heneicosane
50	51.20	-	2.34	-	296	43	123(62),82(61),68(75)	3,7,11,15-Tetra methyl-2-hexadecane-1-ol
51	52.50	-	1.13	-	296	57	113(15),99(25),71(82)	2-Methyl ecosane
52	53.58	-	1.46	-	324	57	113(12),85(55),71(78)	Tricosane

Table 3: GC/MS of EOs using solvent extraction of flowers, leaves and stems of *IC.*

Peak No.	R_t (min.)	Rel. %			Mass data			Compounds
		flowers	leaves	stems	Mol.Wt.	b.p.	Fragments	
1	3.99	1.78	-	1.38	128	43	99(15),71(25,57(92)	n-Nonane
2	4.62	-	0.64	-	136	93	105(9),91(99),77(38)	Thujene
3	4.82	-	1.29	-	136	93	121(12),105(12),77(37)	α-Pinene
4	5.28	-	0.20	-	136	93	121(65),107(28),67(31)	Camphene
5	5.87	-	0.90	-	136	93	121(9),91(40),77(35)	Sabinene
6	6.03	-	0.82	-	136	93	121(15),79(22),69(32)	β-Pinene
7	6.30	-	2.99	-	136	93	107(7),79(14),69(71)	α-Myrcene
8	6.92	1.19	7.32	3.30	136	93	119(10),71(32),65(8)	α-Phellandrene
9	7.21	-	2.31	-	136	93	121(92),105(25),77(35)	α-Terpinene
10	7.55	1.21	12.30	-	134	119	103(8),91(21),77(18)	ρ-Cymene
11	7.63	5.75	30.90	15.38	136	68	107(21),93(75),79(38)	d Limonine
12	7.76	-	2.51	1.64	154	43	139(32),108(59),93(78)	Eucalyptol
13	8.20	-	0.25	-	136	93	121(15),91(24),79(39)	α-Ocimene
14	8.65	2.16	18.57	5.42	136	93	121(26),105(10),77(28)	ɣ-Terpinene
15	9.62	-	0.64	-	136	93	121(85),105(22),79(38)	α-Terpinolene
16	10.31	-	2.05	-	154	71	136(15),93(76),55(59)	Linalool
17	12.33	-	0.73	-	152	95	109(41),81(74),69(40)	Camphor
18	13.02	-	0.25	-	154	112	139(41),97(38),69(72)	L-Menthone
19	13.66	-	1.16	-	154	71	136(15),111(35),93(42)	4-Terpinol
20	13.86	-	0.98	-	152	137	109(24),79(28),69(38)	Dill ether
21	14.54	-	1.96	-	148	148	117(42),105(23),77(31)	Estragole phenyl pro
22	15.91	-	0.97	-	152	109	137(22),95(28),81(39)	α-Cyclocitral
23	16.35	-	0.24	-	196	93	152(8),80(38),43(52)	Linalyl acetate
24	16.74	-	0.41	-	168	43	126(10),107(42),97(53)	Ascaridole
25	17.04	0.41	-	1.44	170	57	113(12),85(59),71(88)	2-Methylundecane
26	22.72	3.18	-	-	204	93	161(32),133(65),105(71)	Iso caryophyllene
27	23.36	1.99	-	-	204	93	189(21),133(72),79(86)	Trans-caryophyllene
28	24.99	-	0.21	1.16	212	57	141(11),85(62),71(92)	Pentadecane
29	25.88	3.88	0.44	3.73	240	71	155(10),85(82),57(93)	Heptadecane
30	26.30	-	0.24	-	204	57	161(29),85(69),71(73)	α-Guaiene
31	27.42	-	0.21	1.08	206	191	111(21),69(52),57(73)	2-Allyl-s-t-butyl quinine
32	27.74	2.74	-	-	268	71	169(9),99(24),57(82)	Nonadecane
33	28.15	2.56	0.22	5	310	57	153(15),111(52),85(49)	Docosane
34	30.05	1.79	-	-	290	79	161(12),109(43),91(65)	Caryophyllene oxide

35	32.29	1.13	-	-	138	43	207(8),136(51),57(81)	Geranyl isovalerate
36	32.67	-	-	1.20	324	43	113(15),65(38),57(78)	8-hexyl heptadecane
37	33.92	-	0.44	2.95	282	71	155(8),113(75),85(75)	2,6,11,15-Tetramethyl hexadecane
38	34.10	5.04	-	-	220	109	202(22),169(38),79(92)	Allo aromadendreneoxide (I)
39	35.01	-	0.6	3.43	242	69	125(35),111(48),57(81)	2-Hexyl-1-Decanol
40	35.36	-	-	1.27	300	43	111(10),83(8),57(15)	Actinobolin
41	35.56	-	0.21	1.83	338	71	113(25),85(72),57(92)	Tetracosane
42	35.68	-	0.19	1.94	298	69	154(10),85(68),57(78)	2-Octyl-1-dodecanol
43	35.88	-	-	1.25	366	57	155(12),85(64),71(80)	3-Ethyl-5-(2-ethyl butyl-octadecane)
44	36.06	-	-	2.71	364	43	153(18),111(42),69(81)	1,4-Dimethyl-2-octadecyl cyclo hexane
45	36.82	-	-	3.08	424	43	165(11),69(22),57(18)	Hexacosyl acetate
46	37.21	-	-	1.71	314	69	208(10),97(35),85(4)	2-Octadecyloxy ethanol
47	37.95	1.24	-	-	256	57	191(18),97(41),72(70)	Tricosane
48	39.50	-	0.86	-	268	43	165(12),71(87),58(91)	6,10,14-Trimethyl-2-pentadecanone
49	40.18	-	-	2.15	390	149	157(10),98(25),57(65)	Dioctyl phthalate
50	41.18	1.46	0.22	-	352	71	209(5),90(42),57(91)	Pentacosane
51	42.45	9.32	-	-	270	74	143(18),87(63),55(25)	Methyl palmitate
52	42.63	9.18	-	6.99	408	71	183(10),85(43),71(52)	Nonacosane
53	43.22	-	-	1.06	314	75	299(71),103(31),55(41)	Hexadecyl oxy,Tri methyl silane
54	43.75	-	-	3.67	450	57	155(10),85(64),71(86)	11-Decyl docosane
55	44.61	6.24	-	-	284	88	157(14),101(36),57(32)	Ethyl palmitate
56	45.10	2.15	-	-	296	55	250(15),110(38),69(78)	Methyl oleate
57	47.71	12.17	-	3.67	506	57	169(5),85(58),71(82)	Hexatriacontane
58	48.88	4.29	-	-	318	74	203(17),91(66),55(48)	Methyl arachidonate
59	49.33	6.59	-	6.25	356	75	327(79),97(71),57(62)	n-Hexadecyl oxy- tri ethyl silane
60	50.95	-	0.77	3.74	281	59	153(10),77(42),43(30)	9-Octadecene amide
61	52.16	6.02	-	-	270	70	152(9),96(15),55(26)	Iso amyl laurate
62	52.56	-	-	1.81	492	57	239(8),85(60),71(78)	Pentatriacontene
63	53.94	3.19	-	-	356	73	239(21),85(43),57(71)	2-Hydroxy-1-methyl propyl stearate
64	59.94	-	-	1.95	436	43	355(12),209(15),57(38)	Ethyl iso allo cholate

Table 4: GC/MS of EOs using SPME of flowers, leaves and stems of *IC.*

Peak No.	Rt (min.)	%			Mass data			Compounds
		flowers	leaves	stems	Mol. Wt.	b.p.	Fragments	
1	3.14	0.55	-	-	153	53	75(10),55(12),52(22)	Imidazole
2	3.15	-	2.84	-	102	44	73(38), 60(95), 55(15)	Valeric acid
3	11.29	0.55	-		198	57	99(18),85(39),71(62)	Tetradecane
4	14.71	2.36	-		178	53	109(15),71(32),58(77)	6-dodecanone
5	16.80	1.31	-	-	184	51	140(19),82(63),57(91)	Dodecanol
6	21.70	-	0.45		204	93	190(22),119(62),53(79)	α-Curcumene
7	21.96	-	5.15	-	204		161(92),134(52),119(82)	Cadinene
8	22.83	-	1.36	-	204	105	189(32),93(95), 79(51)	α-Ylangene
9	22.90	2.69	-	-	258	57	202(18),85(36),71(58)	t-Hexadecan thiol
10	23.54	2.30	-	-	254	57	141(12),85(42),71(79)	2,6,10-Trimethyl pentadecane
11	23.61	-	0.77	-	202	91	131(91),188(45),81(73)	Dehydro-cyclongifolene oxide
12	24.06	-	6.65	-	204	119	161(72),91(52),55(22)	α-Copaene
13	24.16	2.53	1.18	-	204	161	119(48), 93(65),55(48)	α-Guaiene
14	24.36	1.53	1.33	-	202	91	159(85),105(86),77(62)	1(10),4-Aromadendradiene
15	24.93	-	1.58	-	174	159	144(16),115(22),91(31)	1,3,5-Trimethyl-2-(2-butenyl) benzene
16	25.02	1.25	-	-	202	159	174(72), 91(63), 77(59)	α-Vatirenene
17	25.15		3.63	-	204	161	133(18), 105(40), 91(45)	Clovene
18	25.24	1.44	-	-	204	161	189(41),105(38),91(40)	α-Selinene
19	25.49	2.13	-	-	220	58	202(15),109(32),57(58)	8-Cedren-13-Ol
20	25.51	-	1.98	-	204	105	147(15),121(52),105(71)	α-Muurolene
21	25.67	-	1.94		204	119	161(25), 105(50), 91(30)	α-Longipinene
22	25.78	0.81	-	-	204	107	148(35),93(51),53(54)	α-Bulnesene
23	25.91	-	3.19	-	204	161	189(90), 105(57), 91(49)	Cadina-3,9-diene
24	26.02	2.27	-	-	204	189	161(92),134(52),119(82)	Cadinene
25	26.16	-	0.80	-	204	91	161(95),147(20),105(35)	Calarene
26	26.52	17.14	-	-	212	57	99(15), 71(62), 53(96)	Pentadecane
27	26.56		3.19	-	204	43	189(49),137(72),91(92)	α-Chamigrene
28	26.96	3.49	4.35	-	204	91	161(28),106(73),79(88)	Trans caryophyllene
29	27.24	-	0.45	-	204	91	161(96),119(30), 105(32)	Aristolene
30	27.34	-	2.21	-	204	93	119(40), 91(35), 77(23)	Trans-bisabolene
31	27.41	3.12	-	-	268	57	212(8),85(38),71(62)	6-Methyl octadecane
32	28.35	-	4.70	-	204	161	189(19),133(35),67(71)	Gurjunene
33	28.69	-	0.48	-	204	161	161(31),136(50),93(91)	α-Elemene

34	28.80	-	4.32	-	204	91	189(64),105(61),77(58)	α-Neoclovene
35	28.95	0.65	-	-	268	53	189(18),82(42),57(96)	10-methyl-11-tridecane-1-ol- propionate
36	29.27	1.26	-	-	204	105	189(38),161(68),53(60)	(-)-α-Neoclovene
37	30.34	-	9.87	-	204	161	189(64),105(72),91(89)	α-Maaliene
38	30.41	2.67	-	-	204	161	189(53),105(72),91(81)	α- Gurjuenene
39	30.59	-	0.38	-	204	91	175(67), 162(64),131(32)	α-Cedrene
40	30.77	-	7.98	-	204	161	189(22),105(69),81(74)	Isoledene
41	31.01		2.64		220	205	177(25),105(35),57(69)	4-(Phenyl ethynyl) acetophenone
42	31.72	1.21	-	-	204	105	161(82),91(92),53(79)	α-Amorphene
43	31.87		2.94		202	159	133(28),105(19),91(29)	Trans-calamenene
44	31.93	1.67	-	-	202	159	144(15),129(24),105(19)	Cis-calamenene
45	32.16	2.92	-		296	54	182(22),123(49),71(89)	Phytol
46	32.32	-	0.62	-	234	119	133(35),91(52),79(72)	7,10-Pentadecadiynoic acid
47	32.63	0.61		-	236	53	205(30),187(62),91(62)	Tetrahydro isovelleral
48	32.82		3.72	-	180	111	137(48),67(60), 43(98)	Dihydroactinidiolide
49	33.10	-	0.41	-	220	67	205(19),111(75),55(56)	α-Bisabolene epoxide
50	33.46	1.61	-	-	308	53	164(32),107(53),79(75)	Ethyl linoleolate
51	33.60	4.91		-	310	57	141(12), 85(38),71(69)	Docosane
52	34.12	-	2.52	-	220	43	135(35),95(60),55(51)	Trans-longipinocarveol
53	34.34	-	1.34	-	202	187	159(71),145(39),115(28)	1(10),6,8-,Cadinatriene
54	34.37	1.22	-	-	202	187	159(68),141(22),128(35)	Cadina-1(10),6,8-triene
55	34.61	-	-	7.58	358	118	207(41), 97(92), 67(91)	17,21-Dihydroxy Pregna-1,4-diene-3,11,20-trione
56	35.04	-	-	9.92	414	91	396(12),161(52),57(63)	Clionasterol
57	35.20	-	1.92	-	204	81	123(41),91(22),55(19)	α-Bourbonene
58	35.32	-	0.91	-	294	81	220(19),93(52),57(52)	Methyl arachidonate
59	35.54	-	-	26.6	414	120	207(51),162(32),77(62)	3,4,7,8-Tetrakis(t-Butyl) 2 6bis (isopropyl)1,5diazocin
60	35.95	-	-	8.27	398	133	204(25),105(55),57(43)	Benzene,2(1-decyl1-undecenyl)1,4dimethyl
61	36.04		0.47	-	244	115	202(41),141(90),85(91)	Falcarinol
62	36.14	1.52	-	-	346	43	207(14),91(35),55(87)	2-Bromo-octodecanol
63	36.47	0.92	-	-	242	43	207(15),69(49),55(72)	2-Hexadecanol
64	36.94	2.42	-	-	220	43	161(12),109(43),91(65)	Caryophyllene oxide

65	37.15	-	-	0.73	400	77	341(12), 204(41), 96(78)	11à-Hydrozyresibufogenin
66	37.28	-	-	2.09	402	161	356(23),147(51), 57(97)	Prednisolone Acetate
67	37.30	1.99	-		256	57	111(25),85(41),71(65)	2-Methyl 1-hexadecanol
68	37.41	0.79		-	280	43	191(35),91(42),57(54)	Tetra neurin-A-diol
69	37.43	-	2.86	-	206	43	191(92),119(72),55(81)	α-Methylionone
70	37.55	-	0.50	-	350	41	255(11),159(32),93(52)	Chiapin-B
71	38.20	1.06	-	-	324	57	183(10),85(54),71(82)	Tricosane
72	38.54	-	-	6.67	392	77	204(43), 131(49), 55(73)	Ursodeoxycholic acid
73	38.60	-	1.72	-	310	43	153(15),111(52),85(49)	Docosane
74	38.62	1.17	-		352	43	113(12),85(48),71(81)	Pentacosane
75	38.94	-	-	3.37	596	55	288(23), 161(75), 91(83)	Astaxanthin
76	39.01	-	-	7.56	414	43	381(13), 135(54), 62(43)	Stigmast5en3ol,(3á,24S)
77	39.19	-	-	3.28	432	43	396(21), 137(49), 71(42)	24-Methylcholest-7ene-3á,5à,6á-triol
78	42.22	-	-	6.19	436	71	396(52),255(45),95(83)	Ethyl isoallocholate
79	42.29	-	-	2.96	554	55	396(58), 105(54), 85(56)	Rhodopin
80	43.41	3.84	-	-	282	43	193(15),83(35),57(84)	Oleic acid
81	44.00	7.68	-	-	268	43	109(10),71(30),58(52)	6,10,14-trimethyl-2 pentadecanone
82	48.06	-	-	2.85	416	43	355(31), 281(46), 79(85)	Desacetylcinobufotalin
83	49.44	-	-	4.08	428	207	381(23), 207(86), 135(74)	1,25-Dihydroxy vitamin D2
84	51.30	3.88	-	-	416	73	401(65),313(25)193(75)	Calophylloide
85	52.82	4.27	-	-	408	57	141(10),85(42),43(82)	Nonacosane
86	58.64	1.16	-	-	430	73	327(8),193(15),135(9)	1,1,3,3,5,5,7,7,9,9,1 -Dedeca methyl hexa siloxane

Table 5: Summary of all the identified classes of compounds in all methods of *IC* flowers, leaves and stems.

Compounds	MAE %			HD %			SE %			SPME %		
	flowers	leaves	stems	flowers	leaves	stems	flowers	leaves	stems	flowers	leaves	stems
Yield %	0.05	0.02	0.01	0.03	0.01	0.01	0.02	0.03	0.01	-	-	-
Aromatic	-	1.93	7.35	-	-	2.01	1.21	14.47	1.08	0.55	4.22	8.27
Hydrocarbons	5.26	0.99	17.21	2.42	8.59	1.41	35.42	4.36	45.87	48.78	1.72	-
Halogenated hydrocarbons	-	-	-	-	1.03	1.18	-	-	-	1.52	-	-
Monoterpene hydrocarbons	-	7.89	54.89	-	-	15.24	9.1	66.83	24.1	-	-	-
Oxygenated monoterpenes	0.34	-	-	-	4.6	5.71	-	8.09	1.64	-	6.58	-
Sesquiterpene hydrocarbons	16.04	85.7	14.89	22.79	46.04	29.37	5.17	0.24	-	20.13	70.45	-
Oxygenated Sesquiterpenes	76.4	1.29	-	70.08	34.82	34.02	7.96	-	-	7.17	6.61	-
Diterpenes	-	-	-	-	0.32	-	-	-	-	2.92	0.50	7.58
Esters	0.06	-	1.86	0.21	-	4.86	31.21	0.24	7.18	2.26	0.91	8.28
Acids	-	-	-	-	-	-	-	-	-	3.84	3.46	6.67
Nitrogenous compounds	-	-	-	-	-	-	-	0.77	3.74	-	-	26.63
Coumarins	-	-	-	--	-	-	-	-	1.27	3.88	-	-
Silicon compounds	-	-	-	-	-	-	6.59	-	7.31	1.16	-	-
Sulfur compounds	-	-	-	-	-	-	-	-	-	2.69	-	-
Sterols	-	-	-	-	-	-	-	-	-	-	-	14.92
Triterpenoids	-	-	-	-	-	-	-	-	-	-	-	12.77
Tetraterpenoids	-	-	-	-	-	-	-	-	-	-	-	6.33
Total %	98.1	97.8	96.2	95.5	95.4	93.8	96.66	95	92.19	94.9	94.45	92.18

In leaves (Table 5), the monoterpene hydrocarbons obtained by SE and MAE are 66.83% and 7.89%, respectively. It was found that, the EOs obtained by MAE were more rich with sesquiterpene hydrocarbon compounds (85.7%) than in the SPME and HD EOs (70.45% and 46.04%, respectively). Also, the EOs obtained by HD in larger amounts with oxygenated sesquiterpenes compounds (34.82%), and the percentage of oxygenated sesquiterpenes compounds minimized in the EOs obtained by SPME and MAE to 6.61% and 1.29% respectively).

Table 1 showed that, germacrene-D and *Trans*-caryophyllene and δ-Gurjunene were the major components (47.16 %, 13.83% and 6.98% respectively). Additionally, Table 2 revealed that, the major components of the leaves oil using HD were caryophyllene oxide, germacrene-D and *trans*-caryophyllene (23.21%, 17.73% and 14.48%, respectively).

Ogunmoye et al. (2015) stated that, forty-one constituents representing 93.5% of the oil were identified from the GC/MS spectra. Monoterpenes (22.0%), sesquiterpenes (46.5%) and diterpenes (22.5%) were the classes of compounds identified the essential oil, obtained by hydrodistillation from the air dried leaves of *I. batatas*. While, with SE, the main components and appearance of a new other major compounds of EOs obtained were d-limonene, □-Terpinene and ρ-Cymene (30.90%, 18.57% and 12.30%, respectively). At last, the EOs of leaves obtained by using SPME, was found to contain sesquiterpenes compounds (70.45%). The main components of essential oils obtained were α-Maaliene, Isoledene and α-Copaene (9.87%, 7.98% and 6.65%, respectively).

In stems, Table (5) showed that the EOs obtained by MAE were more concentrated in monoterpene hydrocarbons which were present in larger amounts (54.89%) than in the SE (24.1%) and HD (15.24%). By using SE, the obtained EOs of stems were more concentrated in hydrocarbons which were present in larger amounts (45.87%) than in the MAE Eos (17.21%) and HD EOs (1.41%). On the other hand, in MAE (Table 1), limonene, □-Terpinene and *O*-Cymene were the major components (30.34 %, 13.70% and 6.39%, respectively).

It is also noticed that, in Table 5, the nitrogenous compounds were present in stems in both SPME and SE methods (26.63% and 3.74% respectively).

All these results have proved that microwave greatly accelerated the extraction process, but without causing significant affect in the EOs composition. Sesquiterpenes are less valuable than oxygenated compounds in terms of their contribution to the fragrance of the EOs. Conversely, the oxygenated compounds are highly odoriferous and, hence, the most valuable. The greater proportion of the detected compounds and the proportion of oxygenated compounds in MAE EOs were probably due to the diminution of thermal and hydrolytic effects compared with HD, which is time- and energy-consuming. Water is a polar solvent, which accelerates many reactions, especially reactions via carbonation as intermediates. By using these extraction methods, slight differences between the composition of EOs from flowers, leaves and stems of *IC* can be noted as shown in Table 5. The MAE method offers the possibility for better production of the natural aroma of the EOs than that obtained using HD, so, MAE could be a good alternative for the isolation of EOs (Hammouda et al. 2013).

The GC/MS analysis of the FAME of flowers, leaves and stems of *IC* in Table 6 revealed the presence of a mixture of sixteen, fifteen and nineteen fatty acids (saturated and unsaturated), respectively. The saturated fatty acids (SFA) for flowers constitute 44.93%, while for leaves of are 81.91%, and for stems are 66.03%. The unsaturated fatty acids comprise monounsaturated fatty acid methyl esters from flowers, leaves and stems constitute 52.06%, 11.63% and 2.25%, respectively, while, the di unsaturated fatty acid methyl esters constituents are 3.01%, 6.46 and 23.55% respectively. On the other hand, the polyunsaturated fatty acid methyl esters only present in stems (8.17%). The results also proved that, methyl palmitioleate(52.06%), methyl stearate and methyl behenate were recognized as the most common fatty acids present in flowers, while methyl palmitate, methyl stearate and methyl palmitioleate were the major fatty acids in leaves. Also, the most abundant fatty acid methyl ester present in stems were methyl palmitate, methyl linoleate and methyl stearate. Methyl palmitate was the most abundant fatty acid methyl ester present in leaves and stems which gave 36.73%, 28.25% respectively, while in flowers was found as traces where it gave 0.85%. These results are agree with that reported by Sahayaraj *et al*., 2015, where they found, the fatty acids identified from the stems and roots of *IC* contained 8 compounds in which Palmitic acid was the principal constituent (70.61 % and 88.89 %) of the stem and root respectively

Table 6: GC/MS of FAME fraction of flowers, leaves and stems of *IC.*

Peak No.	R_t (min.)	Rel. %			Mol. Wt.	Molecular formula	Compounds
		flowers	leaves	stems			
1	11.88	-	-	0.39	186	$C_{11}H_{22}O_2$	Methyl caprate
2	23.52	0.82	1.38	0.87	242	$C_{15}H_{30}O_2$	Methyl myristate
3	25.80	-	0.24	0.40	296	$C_{19}H_{38}O_2$	Methyl oleate
4	26.18	0.76	0.42	1.05	256	$C_{16}H_{32}O_2$	Methyl pentadecanoate
5	27.78	52.06	11.63	2.25	268	$C_{17}H_{32}O_2$	Methyl palmitioleate
6	28.18	0.85	36.73	28.25	270	$C_{17}H_{34}O_2$	Methyl palmitate
7	31.15	6.03	7.74	2.94	284	$C_{18}H_{36}O_2$	Methyl margarate
8	32.74	3.01	6.46	23.55	294	$C_{19}H_{34}O_2$	Methyl linoleate
9	32.87	-	-	8.17	292	$C_{19}H_{32}O_2$	Methyl linolenate
10	33.49	13.13	17.50	17.02	298	$C_{19}H_{38}O_2$	Methyl stearate
11	35.74	-	-	0.33	312	$C_{20}H_{40}O_2$	Methyl nonadecanoate
12	37.88	2.85	3.97	3.95	326	$C_{21}H_{42}O_2$	Methyl arachidate
13	39.94	0.34	0.39	0.54	340	$C_{22}H_{44}O_2$	Methyl eicosanoate
14	41.97	10.14	8.38	3.30	354	$C_{23}H_{46}O_2$	Methyl behenate
15	43.85	0.41	0.61	0.50	368	$C_{24}H_{48}O_2$	Methyl tricosanoate
16	45.70	3.29	2.74	3.92	382	$C_{25}H_{50}O_2$	Methyl lignocerate
17	45.49	0.41	0.45	0.54	396	$C_{26}H_{52}O_2$	Methyl pentacosanoate
18	49.22	2.18	1.36	1.40	410	$C_{27}H_{54}O_2$	Methyl cerotate
19	52.53	3.05	-	-	438	$C_{29}H_{58}O_2$	Methyl octacosanoate
20	55.64	0.67	-	0.63	466	$C_{31}H_{62}O_2$	Methyl triacontanoate

Table 7: GC/MS of unsap. Fraction of flowers leaves and stems of *IC.*

Peak No.	R_t (min.)	Rel. %			Mol. wt.	Molecular formula	Compound
		flowers	leaves	stems			
1	11.74	-	-	0.14	140	$C_{10}H_{20}$	1-decene
2	25.48	11.91	18.58	23.81	220	$C_{15}H_{24}O$	Butylated hydroxyl toluene
3	27.69	-	1.94	-	220	$C_{15}H_{24}O$	Spathulenol
4	27.80	-	2.15	-	220	$C_{15}H_{24}O$	Caryophylline oxide
5	28.08	0.14	0.50	0.14	226	$C_{16}H_{34}$	n-hexadecane
6	28.87	-	0.43	-	220	$C_{15}H_{24}O$	Ledene oxide
7	29.23	-	0.72	-	220	$C_{15}H_{24}O$	Isospathulenol
8	29.75	-	0.50	-	220	$C_{15}H_{24}O$	Aromadendrene oxide(2)
9	30.91	-	-	0.14	240	$C_{17}H_{36}$	n-heptadecane
10	33.62	0.19	1.38	0.33	254	$C_{18}H_{38}$	n-octadecane
11	35.49	-	2.17	-	278	$C_{16}H_{22}O_4$	2-methyl propyl phthalate
12	37.88	0.12	1.07	0.21	278	$C_{16}H_{22}O_4$	Dibutyl phthalate
13	38.65	0.19	0.8	0.24	268	$C_{19}H_{40}$	n-nonadecane
14	40.13	0.82	0.36	-	282	$C_{20}H_{42}$	2-methyl nonadecane
15	40.93	6.04	-	-	266	$C_{19}H_{38}$	1-nonadecene
16	41.06	3.10	0.91	34.28	282	$C_{20}H_{42}$	n-eicosane
17	41.35	2.71	10.46	0.67	296	$C_{20}H_{40}O$	Phytol
18	43.26	0.34	0.72	0.30	296	$C_{21}H_{44}$	n-heneicosane
19	44.61	1.76	0.63	0.14	296	$C_{21}H_{44}$	2-methyl eicosane
20	45.47	4.10	1.83	-	324	$C_{23}H_{48}$	n-tricosane
21	47.50	0.42	0.62	0.26	338	$C_{24}H_{50}$	n-tetracosane
22	48.78	2.86	0.81	-	366	$C_{26}H_{54}$	n-hexacosane
23	49.55	3.67	1.63	-	394	$C_{28}H_{58}$	n-octacosane

24	50.26	0.87	2.44	1.89	390	$C_{24}H_{38}O_4$	Dioctyl phthalate
25	51.43	0.43	-	0.69	422	$C_{30}H_{62}$	n-triacontane
26	52.59	0.73		-	436	$C_{31}H_{64}$	n-hentriacontane
27	53.34	-	7.22	1.62	450	$C_{32}H_{66}$	n-dotriacontane
28	55.11	12.12	1.32	-	506	$C_{36}H_{74}$	n-hexatriacontane
29	55.22	0.12	0.48	0.43	410	$C_{30}H_{50}$	squalene
30	56.88	-	10.92	8.53	492	$C_{37}H_{76}$	n-heptatriacontane
31	56.99	14.84	2.12	-	534	$C_{38}H_{78}$	n-octatriacontane
32	57.15	6.15	0.24	-	582	$C_{40}H_{82}$	n-tetracontane
33	60.25	12.24	7.75	17.77	618	$C_{44}H_{90}$	n-tetratetracontane
34	60.51	11.14	7.02	-	646	$C_{46}H_{94}$	n-hexatetracontane
35	62.21	2.40	1.32	1.06	400	$C_{28}H_{48}O$	campasterol
36	62.61	-	2.18	2.20	412	$C_{29}H_{48}O$	stigmasterol
37	63.59	-	6.84	5.15	414	$C_{29}H_{50}O$	β-sitosterol
38	64.21	0.23	1.44	-	426	$C_{30}H_{50}O$	α-amyrine
39	64.68	0.19	-	-	442	$C_{30}H_{50}O_2$	Betulene
40	66.67	0.17	-	-	426	$C_{30}H_{50}O$	lupeol

Table 8: GC/MS of acetone insoluble fraction of flowers, leaves and stems of *IC.*

Peak No.	**R_t (min)**	**Rel. %**			**Mol. Wt.**	**Molecular formula**	**Compound**
		flowers	leaves	stems			
1	13.08	22.43	0.89	1.76	144	$C_9H_{20}O$	Nonanol
2	19.22	-	0.3	-	212	$C_{15}H_{32}$	Pentadecane
3	30.35	-	-	0.44	200	$C_{13}H_{28}O$	Tridecanol
4	30.91	-	0.15	-	240	$C_{17}H_{36}$	Heptadecane
5	33.61	-	0.15	-	254	$C_{18}H_{38}$	Octadecane
6	35.93	68.22	1.20	79.84	242	$C_{16}H_{34}O$	Hexadecanol
7	38.46	-	-	9.66	284	$C_{19}H_{40}O$	Honadecanol
8	45.38	-	-	7.72	326	$C_{22}H_{46}O$	Behenic alcohol
9	49.66	9.35	-	-	396	$C_{27}H_{56}O$	Heptacosanol
10	51.90	-	49.53	-	478	$C_{34}H_{70}$	Tetratriacontane
11	53.28	-		-	492	$C_{35}H_{72}$	Pentatriacontane
12	56.71	-	1.65	0.58	522	$C_{36}H_{74}O$	Hexatriacontanol
13	57.50	-	0.13	-	592	$C_{36}H_{74}O$	Hentetracontanol
14	61.73	-	46.00	-	618	$C_{44}H_{90}$	Tetratetracontane

Table 9: TP and TF Contents of flowers, leaves and stems of *IC.*

	TPC mg Gallic/1g herb		**TFC mg rutin/1g herb**	
	Mean	SD	Mean	SD
Flowers	2.99	±0.288	2.77	± 0.191
Leaves	7.24	±0.288	5.06	± 0.004
Stems	2.88	± 0.292	2.65	± 0.244

Table 10: Antioxidant activity of alcoholic extracts of *IC* different organs.

Samples	mg/ml DPPH				
	0.5	1	1.5	2	IC_{50}
Flowers	11.01	19.56	29.63	43.68	2.43
Leaves	25.06	44.26	66.86	82.32	1.11
Stems	10.77	13.82	15.93	24.59	7.35

The data in Table 7 showed that, the unsapoifiable matter of *IC* flowers, leaves and stems contain the oxygenated compounds, reached to 18.6%, 52.24 and 34.78%, respectively, while the non-oxygenated compounds were 81.4%,47.76% and 65.22%, respectively.

Also, the unsaponifisable fractions of flowers, leaves and stems consist mainly of a mixture of hydrocarbons from C_{10} to C_{46} representing 81.4%, 51.87% and 65.01%, respectively, and phthalate compounds which representing 0.99%, 5.68 and 2.1%, respectively. In addition to a sterol fraction in which campasterol present in flowers 2.40%, leaves 1.32% and stems 1.06%, while, stigmasterol and β-sitosterol present in leaves and stems only. where β-sitosterol was higher in leaves (6.84%) than in stems (5.15%). On the other hand, leaves and stems contain nearly the same amounts of stigmasterol (2.18 % and 2.20% respectively).

The GC/MS of acetone insoluble matters of flowers, leaves and stems of *IC* (Table 8) showed that, it is a mixture of hydrocarbons and fatty alcohols. Flowers contain 3 fatty alcohols in which hexadecanol were the main fatty alcohol (68.22%). The leaves contain 5 hydrocarbons representing 96.13%, tetratriacontane was the main hydrocarbon (49.53%), and 4 fatty alcohols representing 3.87% with hexatricontanol as the main fatty alcohol(1.65%). The stems contain 6 fatty alcohols representing 100% of acetone insoluble fraction, hexadecanol was the main fatty alcohol (79.84%).

The phenolic compounds were isolated from the ethyl acetate of the flowers methanolic extract which were identified as C-I: umbelliferon, C-II: kaempferol 3-*O*-glucoside and C-III: kaempferol using cochromatographic and spectroscopic data (Jizhong et al., 2006; Ambiga et al., 2007; Sahayaraj and Ravi, 2008).

The results in Table 9 showed that the leaves have a high TPC and TFC where it gave TPC of 7.24 mg GAE ± 0.288 / g dry sample, which was higher than that found for flowers (2.99 mg GAE ± 0.288 / g dry sample) and stems (2.88 mg GAE ± 0.292 / g dry sample). On the other hand, our results showed that the TFC of leaves was about 5.06 mg rutin ± 0.004 / g dry sample, while that of flowers, was of 2.77 mg rutin ± 0.191 / g dry sample and for stems 2.65 mg rutin ± 0.244 / g dry sample. These findings are not agree with that reported by Khatiwora et al. 2010, where they reported that, the TFC of *IC* leaves, stem and flower were found ranging from 84 to 422 mg quercetin equivalent/g of dry sample. The TFC of the flowers was quite high compared to that of the leaves and the stem. Also, the values were found between 45 to 73 mg catechol equivalent /g dry sample. The flowers contain the maximum and the stem contains the minimum amounts of phenolic compounds. This is may be due to the difference in locality and the weather conditions.

Antioxidant activity:

Free radical scavenging properties of *IC* different organs are presented in Table10, the flowers, leaves and stems alcoholic extracts gave IC_{50} (2.43 µg/ml, 1.11 µg/ml and 7.35µg/ml) indicating that, the methanolic extract from the leaves showed the highest antioxidant activity. The obtained results are disagree with that reported by Fatima *et al.,* in 2014, where they found that, the flowers of *IC* are more abundant in anti-oxidant phytoconstituents. Also, Hasan et al., in 2015 stated that, the methanolic extract from the flowers showed the highest antioxidant activity (using DPPH radical scavenging test). Diversity and sampling of collection sites (regarding abiotic and biotic factors), as well as the method of extraction may lead to variations in these results.

CONCLUSION

The microwave extraction method is the best method for extraction of essential oil constituents. The flavonoidal constituents were isolated from the ethyl acetate of the flowers methanolic extract which were identified as: umbelliferon, kaempferol 3-*O*-glucoside and kaempferol. Also, the results showed that, the leaves have a high TPC and TFC more than that found for flowers and stems. The leaves alcoholic extract exhibited the highest activity using DPPH assay (IC_{50} =1.11 mg/ml).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Abdelshafeek K.A., putting the idea, designed the experiments, separate pure compounds and collect results, reviewed the manuscript. Abdallah W.E., share in putting the idea, designed the experiments, collection the plant organs, make the laboratory and store experiments, extract with different methods, separate pure compounds and collect results, writing the research, reviewed the manuscript. Osman A.F., share in plant organs extraction, isolation and reviewed the manuscript. El Gendy A.G. analyzed different extracts using GC/MS, determined TPC, TFC and evaluate antioxidant activity. Omer E.A. auditing, writing the research and reviewed the manuscript. All authors read and approved the final version.

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