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TOTAL PHENOLIC AND FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF LANTANA CAMARA AND CUCURBITA PEPO (SQUASH) EXTRACTS AS WELL AS GC-MS ANALYSIS OF LANTANA CAMARA ESSENTIAL OILS

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ABSTRACT

This study aimed to determine total phenolic and flavonoid contents as well as evaluate antioxidant activity of the defatted methanolic extracts of flowers, leaves, stems and certain fractions (dichloromethane, ethyl acetate, n-butanol and water residue) derived from the defatted methanolic extract of *Lantana camara* and *Cucurbita pepo* (Squash) leaves besides, the essential oils of *L. camara* leaves and flowers by using (DPPH, ABTS and TAC assay). The chemical composition of *L. camara* essential oils was identified by GC-MS analysis. The results exhibited that the defatted methanolic extracts of *L. camara* (flowers, leaves and stems) have higher phenolic and flavonoid contents as well as antioxidant activity than *C. Pepo* (Squash) parts. Also, the ethyl acetate fraction of *L. camara* has the highest phenolic and flavonoid contents as well as the highest antioxidant activity (DPPH; SC_{50} = 11.82±0.21 µg/ mL, TAC; 290.96±6.71mg eq. ascorbic acid /g extract, ABTS; 94.03±0.25mm Trolox® eq. / 100g extract). There is a positive correlation between the total phenolic and flavonoid contents and the antioxidant activity of the tested extracts or fractions. GC-MS analysis of essential oil of *L. camara* leaves and flowers revealed the presence of 47 compounds in leaves and 40 compounds in flower. The major compounds in the leaf oil were 7(11) -selinen-4α-ol (14.50%) and cedrenol

(6.50%) whereas, the major compounds in flower oil were cedrenol (10.71%) and farnesyl acetone (7.15%).

In Summary: the defatted methanolic extracts of *L. camara* and *C. pepo* and the ethyl acetate fractions can be recommended as natural antioxidant agents.

KEYWORDS: antioxidant activity, *Cucurbita pepo* (Squash)**,** essential oil, flavonoids, *Lantana camara*, phenolic.

INTRODUCTION

The Free radicals role is well established in disease pathology and is known to be included in acute and chronic diseases.[1] Our bodies have an inherent antioxidant mechanism and many biological functions as anti-aging, anti-carcinogenic and anti-mutagenic responses create from this property.[2, 3] An imbalance between the reactive oxygen species and the inherent antioxidant capacity of the body causes numerous disorders and diseases.[1, 4] Usually, before the free radicals attack their targets in biological cells, antioxidants stabilize or disrupt them.[4] Natural Antioxidant agents have attracted the interest due to their free radical scavenging ability.[5] Concerning on this ability, the recent interest increased use natural antioxidants in food, cosmetics, and pharmaceutical products because they possess multifacetedness in their multitude and magnitude of activity and correct the imbalance in the human body.[6, 7] The use of medicinal plants with a high level of antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages.[8] Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds as phenols, flavonoids, tannins, vitamins and carotenoids which play an important role in health protection from the risk of many diseases.[9, 10]

Lantana camara L. (family *Verbenaceae*) is a tree native from Africa and America and has been cultured as an ornamental plant in other countries.[11] It is listed as one of the important medicinal plants of the world.[12] It has been used to possess various biological activities such as anti-protozoal, anti-inflammatory, antibacterial and antioxidant activity.[13, 14] The essential oil of *L. camara* showed anti-inflammatory, anti-bacterial and antimicrobial activities.[15, 16] Its therapeutic potential is due to the presence of bioactive compounds such as flavonoids, flavones, isoflavones, coumarins, anthocyanins, lignans, isocatechins, catechins, tannin, alkaloids, saponins and triterpenoids.[17]

Cucurbita pepo L. (Squash) is one of the important vegetables grown in Egypt. It belongs to Cucurbitaceae family which use in various traditional medicines as antibacterial, anti-diabetic, antitumor, immune-modulation, anti-hypertensive, anti-inflammation, anti-hypercholesterolemia, and anti-parasitic.[18] It is rich in amino acids, polysaccharides, carotenoids, fatty acids, minerals terpenoids, cucurbitosides, carotenoids and cucurbitacin glycosides.[19, 20]

Therefore, the current study was designed to determine the total phenolic and flavonoid contents as well as evaluate the antioxidant activity of the defatted methanolic extracts of (flowers, leaves and stems) and certain fractions derived from the two methanolic extracts of the leaves of *L. camara* and *C. pepo*. Also, the chemical composition of the essential oils of *L. camara* leaves and flowers was identified by using GC-MS analysis.

MATERIAL AND METHODS

Chemicals

DPPH (1, 1diphenyl-2-picryl hydrazyl radical), ABTS (2-2`azinobis (3-ethylbenzthiazoline-6-sulphonic acid), and Folin- Ciocalteu reagent were purchased from Sigma –Aldrich (Germany). sodium hydrogen phosphate and Potassium persulphate sodium nitrite, sodium hydroxide, ammonium molybdate, sodium bicarbonate and aluminum chloride were purchased from Merck (Germany).Gallic acid, rutin, and ascorbic acid were purchased from Sigma –Aldrich (USA). α-tocopherol (Vitamin E) was purchased from Sigma –Aldrich (England).

Plant materials

Lantana camara plant was collected from the garden of Theodor Bilharz Research Institute, Giza, Egypt. Whereas, *C. pepo* (Squash) plant was collected from local markets in Giza, Egypt. The two plants were identified by Dr. Rim Samir Hamdy, Professor of plant taxonomy, Faculty of Science, Cairo University, Giza, Egypt. The voucher specimens of the plants were stored in Medicinal Chemistry Department, Theodor Bilharz Research Institute. The flowers, leaves and stems of each plant were separately washed with distilled water and dried in air at room temperature. The dried parts were ground using an electric mill and the fine powders were stored in dried containers for the extraction process.

Extraction and fractionation process

500 grams of dried powders of the flowers , leaves and stems of *L. camara* and *C. pepo* (Squash) were separately extracted with 85 % methanol then evaporated under reduced pressure using rotatory evaporator (BUCHI, Switzerland) till dryness. The dried methanolic extract of each part was defatted with petroleum ether. The aqueous defatted methanolic extract of the leaves of each plant *L. camara* and *C. pepo* (Squash) was fractionated using dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and n-butanol (n-BuOH). Each fraction was dried using rotatory evaporator and the water residue was completely dried. The methanolic extracts and the fractions were kept away from moisture for the present study.

Essential oil Extraction

3 Kg of each fresh leaves and flowers of *L. camara* were separately cut into small pieces and submitted to hydro distillation process using a Clevenger-type apparatus. The plant materials were immersed in distilled water in a 5 L glass flask. The Oils recovered and stored at 4°C for GC-MS analysis.[21]

Preliminary phytochemical screening

The preliminary phytochemical screenings of the defatted methanolic extracts of the flowers, leaves and stems of *L. camara* and *C. pepo* (Squash) were carried out to determine qualitative contents of carbohydrates, alkaloids, saponins, flavonoids, tannins, sterols, terpenes and anthraquinones. The experiments were performed according to methods that reported by.[22, 23]

Total phenolic content

The phenolic content of the tested extracts was determined using a spectrophotometric method described by **[24].** 0.5mL of each extract (250 µg/mL); 2.5 mL of Folin- Ciocalteus reagent (10 %) dissolved in water and 2.5mL$NaHCO_3$ (7.5%). Blank sample contains 0.5mL MeOH, 2.5 mL of Folin-Ciocalteus reagent (10 %) dissolved in water and 2.5 mL $NaHCO_3$ (7.5%). All mixtures were shaken and incubated at 45°C for 45 min. the absorbance was recorded at 765 nm against a blank sample, and gallic acid was used as the standard. The experiment was carried out in triplicate. The total phenolic content was expressed in terms of gallic acid equivalent (GAE) per gram dry weight of the extract.

Total flavonoid content

The content of flavonoids of the tested extracts was determined using a colorimetric assay reported by [25]. 0.5 mL of each extract was mixed with 2 ml distilled water and 150 µL of $NaNO_2$ (5%) for 6 min, then 150 µL of $AlCl_3$ (10%) was added and allow to stand for 5 min then added of 2mL NaOH (4%) and adjusted to 5mL with 200 µL distilled water. The mixture was incubated at room temperature for 15 min. the absorbance was measured at 510nm against a blank sample, and rutin was used as the standard. The total flavonoid content was estimated as mg rutin equivalents (RE) per gram extract. The experiment was carried out in triplicate.

Antioxidant Assays

DPPH radical scavenging activity

The antioxidant activity of the tested extracts was evaluated using DPPH free radical scavenging method.[26] 2 mL of each extract concentration solution was mixed with 2 mL of DPPH in MeOH (0.1 m mol /L). The control contained solvent and DPPH except for the extract. The mixtures were shaken well and kept in dark for 30 min at 37˚C.The absorbance was measured at 517nm. Ascorbic acid and vitamin E were used as standards and the experiment was carried out in triplicate. The DPPH scavenging activity of the extracts was calculated from this equation.

Scavenging activity % = [(control absorbance - sample absorbance) / (control absorbance)] × 100

Total antioxidant capacity (TAC) assay

The total antioxidant capacity of the tested extracts was determined by [1, 27]. An aliquot of 0.1mL of sample (200µg/mL) solution was mixed with 1mL of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Blank was prepared to contain 1mL of the reagent solution and the appropriate volume of the same solvent used for the samples. The tubes were capped and incubated at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against the blank. The experiment was repeated for 3 times. The antioxidant activity of the extracts was expressed as the number of equivalents of mg ascorbic acid (AAE) per gram extract.

ABTS radical - scavenging activity assay

The plant extracts can able to quench $ABTS^{\cdot+}$ (2-2`azinobis (3-ethylbenzthiazoline-6-sulphonic acid) in comparison to Trolox® [28]. The concentrated reagent solution was prepared by dissolving 9.6 mg ABTS in 2.5mL water and then adding 110µL of a solution made by dissolving 37.5 mg of potassium persulphate ($K_2S_2O_8$) in 1mL of water to produce $ABTS^{\cdot+}$ radical cation. The stock solution was kept in the dark room for 12-16 hours before use; for study the $ABTS^{\cdot+}$solution was diluted to an absorbance value between 0.7 and 0.8 at wavelength 734 nm. Subsequently, 100µL of aqueous or alcoholic plant extract (according to solubility) was added to 1 mL of work solution, and it was measured exactly after 2.5 min. Also, an appropriate solvent blank was measured. Calibration curve for $ABTS^{\cdot+}$was obtained using Trolox® (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) as standard. The experiment was carried out in triplicates. Results were expressed in terms of mm Trolox® equivalent per 100 g dry weight of plant extract.

Gas chromatography-mass spectrometry (GC-MS) analysis

Essential oils of the leaves and flowers of *L. camara* were injected to GC-MS technique. The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 Elm film thickness). The carrier gas was helium with the linear velocity of 1 ml/min. The injector and detector temperatures were 200º C and 250º C, respectively. Injection mode, split; split ratio 1: 10, volume injected 1µl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250º C, and acquisition mass range 50–600. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY libraries as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

Statistical analysis

The statistical analysis was performed using SPSS (16) software. The results were given as means ± standard deviation (SD) and all experimental analyses were carried out in triplicate.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

In the present study, the preliminary phytochemical screening of the defatted methanolic extracts of leaves, stems and flowers of *L. camara* and C. *pepo* (Squash) was carried out. The results in **Table (1),** revealed the presence of sterols, terpenoids, flavonoids, carbohydrates, glycosides and saponins in the different parts of the two plants whereas the anthraquinones were absent. Alkaloids are present only in the different parts of *C. pepo* (Squash) and leaves of *L. camara*. Also, the defatted methanolic extracts of the flowers and leaves of *L. camara* had a higher amount of flavonoid compounds than that present in *C. pepo* (Squash). The high amount of carbohydrates is present in the leaves of the two tested plant extracts. These results are in agreement with previous studies on other plant species.[29, 30]

Table (1): Phytochemical screening of the defatted methanolic extracts of leaves stems and flowers of *L. camara* and *C. pepo* (Squash)

Chemical Category	Name of Test	*L. Camara*			*C. Pepo*		
		Leaves	Stem	flower	Leaves	Stem	flower
Carbohydrates	Molisch's test	++	+	+	++	+	+
Alkaloids	Wagner's test	+	-	-	+	+	+
Flavonoid	Shinoda's test	++	+	++	+	+	+
Saponin	Froth test	++	++	+	+	+	-
Tannins	Ferric chloride test	++	-	+	++	-	+
sterols and or/ triterpenes	Libermann-Burchard test	+++	++	+	++	++	+
Anthraquinones	Borntrager's test	-	-	-	-	-	-

(+++): high amount, (++): moderate amount, (+): small amount, (-): Absent.

Phenolic and flavonoid contents

The results in **Table (2)** exhibited that the defatted methanolic extracts of the flowers and leaves of *L. camara* have higher phenolic and flavonoids contents [180.3±0.4 (mg GAE eq. /g extract) and 99.7±0.9 (mg RE eq. /g extract)] respectively for flowers and [148.97±2.1) mg GAE eq. /g extract) and 63.767±1.2 (mg RE eq. /g extract)] respectively for leaves than that present in the defatted methanolic extract of *C .pepo* (Squash). Also, the total phenolic and flavonoids contents of the stems were lowest in the different parts of the two plants under investigation. The ethyl acetate fraction derived from the defatted methanolic extract of *L. camara* had the highest phenolic and flavonoids contents [301.5±3.5 (mg GAE/g extract) and 126.17±1.3 (mg RE /g extract)] respectively. Also, the ethyl acetate fraction derived from

defatted methanolic extract of *C. pepo* (Squash) contains higher phenolic and flavonoid contents [99.70±0.60 (mg GAE/g extract) and 79.30±1.51 (mg RE/g extract)] respectively than other fractions from this plant.

It has been reported that the phenolic and flavonoid compounds consider high potent antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species.[31] Therefore, the high phenolic and flavonoid contents in the defatted methanolic extract and ethyl acetate fraction of the two plant will be recommended that the two plants are nature sources of antioxidant agent especially *L. camara* plant.

***In vitro* antioxidant properties**

DPPH radical-scavenging activity

The DPPH method is widely used to test the ability of plant extracts or compounds to act as free radical scavengers or hydrogen donors, so DPPH is consider a rapid assay for evaluation of antioxidant activity of the plant extract.[32] In the present study, The results in **Table (2)** showed that, the DPPH scavenging activity of the defatted methanolic extracts of the flowers, leaves and stems of *L. camara* and C. pepo (Squash) have antioxidant activity and the activity of the different parts of *L. camara* is higher than *C. pepo* (Squash) as follows: [(SC_{50}= 46.47±0.80, 47.34 ±0.60, 77.43 ±1.42 µg /mL)] respectively for *L. camara* and [584.81±6.96, 625.02 ±8.80 and 667.46±5.40 µg/mL)] for *C. pepo* (Squash) respectively. Also, the ethyl acetate fraction derived from the defatted methanolic extract of leaves of *L. camara* as shown in **Table (3)** has higher active (SC_{50}=11.82±0.21µg/mL) than vitamin E (SC_{50}=15.34±0.80 µg/mL) and the ethyl acetate fraction of C. *pepo* (Squash) (SC_{50}=149.74±2.80 µg/mL) whereas, it is less active than ascorbic acid (SC_{50}=7.67±0.30 µg/mL). The results in **Table (3)** also revealed the butanolic, water residue and dichloromethane fractions of *L. camara* have higher potent antioxidant activity (SC_{50}=28.3±0.26, 76.48±0.96 and 114.04±0.64µg/mL) than the same fractions derived from the leaves of *C. pepo* (Squash) (SC_{50}=367.45±2.97, 650.91±5.44 and 661.96±4.11 µg /mL) respectively. These results reflected that the ethyl acetate fraction derived from the defatted methanolic extract of *L. camara* has high scavenger activity due to its high phenolic and flavonoid contents and these results are in agreement with previous studies carried by.[33, 34]

Total antioxidant capacity (TAC) assay

It has been reported that the total antioxidant capacity is based on the reduction of Mo (VI) to Mo (V) by plant extract or fraction and this indicated by the formation of green phosphate

Mo (V) complex at acidic medium.[35] The results in the **table (2)** showed that, the defatted methanolic extracts of flowers and leaves of *L. camara* has a higher total antioxidant capacity (175.33±1.3 and 173.6±3.2 mg AAE/g extract) respectively than the defatted methanolic extract of flowers and leaves *C. pepo* (136.03±1.5 and 113.83±1.70 mg AAE/ g extract) respectively . On the other hand, each ethyl acetate fraction derived from the defatted methanolic extracts of *L. camara* and *C. pepo* leaves as shown in **Table (3)** exhibited the highest antioxidant capacity (290.96±6.71 and 210.96±1.7 mg AAE/g extract) respectively . Also, the butanolic fractions of *L. camara* and *C. pepo* possess moderate total antioxidant capacity (204.2±7.47 and 179.13±1.16 mg AAE/g extract) respectively. These data indicated that high total antioxidant capacity (TAC) of the plant extract or fraction is correlated with the high amount of the phenolic and the flavonoid content of plant extract. These results are in agreement with many previous studies on other plant extracts.[36, 37, 38]

ABTS radical - scavenging activity

ABTS radical scavenging activity is also fast and effective assay for evaluation of the antioxidant of plant extract. $ABTS^{\cdot+}$ is a protonated radical has characteristic absorbance maxima at wavelength 734 nm and this can decrease with the scavenging of the proton radicals.[39] It has been reported that the scavenging of the $ABTS^{\cdot+}$radical by the extracts is much higher than that of DPPH radical.[32, 40] In this study, the defatted methanolic extracts of the flowers and leaves of *L. camara* as shown in the **table (2)** exhibited a high $ABTS^{\cdot+}$ radical-scavenging activity (58.32±0.7and 55.93±0.5 mm Trolox ®eq. / 100g extract) respectively. Moreover, the results in **Table (3)** showed that the ethyl acetate fractions derived from the defatted methanolic extract of each tested plant *L. camara* and *C. pepo* exhibited high $ABTS^{\cdot+}$radical scavenging activity (94.03±0.25 and 57.90±0.30 mm Trolox® eq./ 100g extract) respectively. These data exhibited that $ABTS^{\cdot+}$radical scavenging activity is correlated with high phenolic and flavonoid contents of plant extract. Therefore, there is a strong positive correlation between the total phenolic and flavonoid contents of the tested plant extract or fraction and their antioxidant properties. These results are in agreement with the previous results on many fruits, vegetables and grain products.[41, 42]

The results of DPPH radical-scavenging, total antioxidant capacity (TAC) and ABTS radical-scavenging activities of the essential oils of the leaves and flowers of *L. camara* as shown in **Table (3)** exhibited that the essential oils of this plant have low antioxidant activity. This result agreed with[43] who reported the low antioxidant activity of *L. camara* leaf essential oil

regarding the composition of leaf essential oil. Otherwise, this result confirmed that higher antioxidant activity is correlated to the presence of the phenolic compounds.[44]

Table (2): Total phenolic and flavonoid contents as well as DPPH, TAC and ABTS activities of the defatted MeOH extracts of flowers, leaves and stems of L. *camara* and *C. Pepo* (Squash)

Extract		Total phenolic (mg GAE eq. /g of extract)	Total flavonoids (mg RE eq. /g extract)	DPPH free radical scavenging activity SC_{50} (µg/ ml)	TAC (mg eq. ascorbic acid /g extract)	ABTS (m mol Trolox eq. / 100g extract)
L. Camara	flower	180.3±0.40	99.7±0.90	46.47±0.80	175.33±1.30	58.32±0.70
	Stems	57.283±0.98	42.633±0.40	77.43±1.40	126.4±3.20	30.83±0.40
	leaves	148.97±2.10	63.767±1.20	47.336±0.60	173.6±3.20	55.93±0.50
C. Pepo	flower	42.30±0.44	33.01±1.25	584.81±6.90	136.03±1.50	35.03±0.45
	Stems	33.77±0.60	22.60+1.80	667.46±5.40	76.80±1.30	18.10±0.26
	leaves	39.63±0.55	30.60±0.96	625.02±8.80	113.83±1.70	28.13±0.35
Ascorbic acid		--	--	7.67±0.30	--	--
Vit. E		--	--	15.34±0.80	--	--

Values expressed as means ± standard deviation (SD).

Table (3): Total phenolic and flavonoid contents as well as DPPH, TAC and ABTS activities of certain fractions derived from the defatted methanolic extracts of leaves of *L. camara* and *C. pepo* (Squash) **and**

Plant /fraction		Total phenols (mg GAE eq. /g of extract)	Total flavonoids (mg RE eq. /g extract)	DPPH free radical scavenging activity SC_{50} (µg/ ml)	TAC (mg eq. ascorbic acid /g extract)	ABTS(m mol Trolox eq. / 100g extract)
L. Camara	CH_2Cl_2 Fr.	50.7±1.18	37.27±0.76	114.04± 0.64	88.067±4.52	26±1.18
	EtOAC Fr.	301.5±3.5	126.17±1.3	11.82±0.21	290.96±6.71	94.03±0.25
	BuOH Fr.	168.87±2.1	104.2±2.05	28.3±0.26	204.2±7.48	80±1
	H_2O Res.	69.67±3.5	46.63±0.76	76.48±0.96	150±5.54	41.73±0.75
	Leaves oil	__	__	876.27±4.9	36.17±4.2	11.13±1.36
	Flower oil	__	__	>1000	21.06±7.1	7.08±1.24
C. pepo	CH_2Cl_2 Fr.	24.03±0.60	11.53±1.46	661.96±4.11	68.53±1.01	10.7±0.46
	EtOAC Fr.	99.70±0.60	79.30±1.51	149.74±2.80	210.96±1.70	57.90±0.30
	BuOH Fr.	62.37±0.35	49.47±1.19	367.45±2.977	179.13±1.16	39.23±0.15
	H_2O Res.	29.83±0.55	22.27±0.91	650.91±5.44	92.60±1.10	15.73±0.64

Values expressed as means ± standard deviation (SD)

GC-MS analysis of essential oil of leaves and flowers of *L. camara*

GC-MS analysis of each essential oil of leaves and flowers of *L. camara* was carried out and the results are represented in the **Table (4)**. The identification of the components of each essential oil was performed by their retention time (RT), molecular formula (MF), molecular weight (MW), concentration (%) and mass fragmentation pattern. These compounds are listed according to their retention times. The present data showed that the identified compounds in the essential oil of the leaves are 47 compounds whereas in flowers are 40 compounds. The percent of total identified compounds in leaves and flowers of *L. camara* are 98.37 % and 90.35%, respectively. The identified compounds in both leaves and flowers can be classified into major nature groups such as monoterpenes, sesquiterpenes, and fatty acids. The major compounds detected in essential oil of leaves *L. camara* were 7(11)-selinen-4α-ol (14.5%), cedrenol (6.5%), 4a,7-methano-4a H-naphth [1,8a-b]oxirene, octahydro-4,4,8,8-tetramethyl (6.5%), linoleic acid (6.3%), δ-cadinene (5.8%), spathulenol (5%) and guaiol (5%). On another hand, the total identified compounds in the essential oil of plant flowers were cedrenol (10.71%), farnesyl acetone (7.15%), germacron (5.21%), clovane (5.08%) and methoxyeugenol (5.06 %) **fig(1).** The results of GC-MS analysis revealed that the chemical constituents of the essential oil of the leaves of *L. camara* are almost matched with the chemical constituents of the essential oil of the flowers but they significantly differed with the percentage of their chemical composition. These results are in agreement with previous studies reported by.[45, 46, 47]

Table (4): Results of GC-MS analysis of essential oils of the leaves and flowers of L. camara.

Peak no.	Components	MF	MW	t_R	Total % of Leaves	Total % of Flowers
1	Pyrocatechitol	$C_6H_{12}O_2$	116	5.71	0.2	—
2	7-Norbornanol	$C_7H_{12}O$	112	7.4	0.18	—
3	p-Menth-1-en-9-al	$C_{10}H_{16}O$	152	7.58	0.2	—
4	D-Limonene	$C_{10}H_{16}$	136	7.8	0.2	—
5	β-Santalene	$C_{15}H_{24}$	204	7.9	0.18	—
6	2,6-dimethyl-2,6-Octadiene-1,8-diol	$C_{10}H_{18}O_2$	170	8.08	0.2	0.26
7	5-methyl-o-Anisidine	$C_8H_{11}NO$	137	8.2	0.25	—
8	(E)-p-2,8-menthadien-1-ol	$C_{10}H_{16}O$	152	8.76	0.4	—
9	N-methyl-p-Anisidine	$C_8H_{11}NO$	137	11.3	0.7	0.48
10	Nootkatone	$C_{15}H_{22}O$	218	11.55	0.4	0.45
11	α-Longipinene	$C_{15}H_{24}$	204	11.6	4.8	0.33
12	D-Carvone	$C_{10}H_{14}O$	150	11.7	3	1.24

13	Cyclosativene	$C_{15}H_{24}$	204	11.9	4.5	0.97
14	Cedrenol	$C_{15}H_{24}O$	220	12.08	6.5	10.71
15	7(11)-Selinen-4α-ol	$C_{15}H_{26}O$	222	12.25	14.5	2.58
16	Linoleic acid	$C_{18}H_{32}O_2$	280	12.3	6.3	3.13
17	α-Gurjunene	$C_{15}H_{24}$	204	12.36	4.2	4.55
18	2H-2,4a-Methanonaphthalen-8(5H)-one, hexahydro-1,1,5,5-tetramethyl-	$C_{15}H_{24}O$	220	12.488	2.7	3.12
19	4a,7-Methano-4aH-naphth[1,8a-b]oxirene, octahydro-4,4,8,8-tetramethyl-	$C_{15}H_{24}O$	220	12.66	6.5	3.14
20	Spathulenol	$C_{15}H_{24}O$	220	12.67	5	3.51
21	δ-Cadinene	$C_{15}H_{24}$	204	12.769	5.8	3.36
22	Guaiol	$C_{15}H_{26}O$	222	12.83	5	1.76
23	2-dimethylamino-Phenol	$C_8H_{11}NO$	137	12.93	3.5	1.61
24	Arachidonic acid methyl ester	$C_{21}H_{34}O_2$	318	12.98	4	2.96
25	Germacron	$C_{15}H_{22}O$	218	13.07	2	5.21
26	Clovane	$C_{15}H_{26}$	206	13.13	3.7	5.08
27	p-Mentha-1,8-dien-7-yl acetate	$C_{12}H_{18}O_2$	194	13.23	2	1.73
28	Farnesyl acetone	$C_{18}H_{30}O$	262	13.3	0.8	7.15
29	2-dimethylamino-Phenol	$C_8H_{11}NO$	137	13.38	0.5	1.73
30	Methoxyeugenol	$C_{11}H_{14}O_3$	194	13.45	0.86	5.06
31	Corodane	$C_{10}H_{14}O$	150	13.7	3.2	1.32
32	(E)-p-2,8-Menthadien-1-ol	$C_{10}H_{16}O$	152	13.72	0.45	1.86
33	Linolenic acid	$C_{18}H_{30}O_2$	278	13.84	0.29	1.28
34	β-Santalol	$C_{15}H_{24}O$	220	13.9	0.35	1.28
35	Gentisic acid, methyl ester	$C_8H_8O_4$	168	14.098	0.25	1.21
36	Methyl 6,11-octadecadienoate	$C_{19}H_{34}O_2$	294	14.17	0.3	1.14
37	8-Hydroxylinalool	$C_{10}H_{18}O_2$	170	14.53	0.25	1.13
38	octahydro-4,7-Methano-1H-inden-1-ol	$C_{10}H_{16}O$	152	14.6	0.3	1.41
39	3-(2,6,6-trimethyl-1-cyclohexen-1-yl) propanal	$C_{12}H_{19}DO$	181	14.8	0.2	1.14
40	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	15.02	0.85	1.13
41	11,13-Tetradecadien-1-ol acetate	$C_{16}H_{28}O_2$	252	15.2	0.35	0.67
42	cis-10-Nonadecenoic acid	$C_{19}H_{36}O_2$	296	15.33	0.54	1.12
43	trans-2-Octenoic acid	$C_8H_{14}O_2$	142	15.46	0.2	1.28
44	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	15.7	0.77	0.82
45	11-aminoundecanoic acid	$C_{11}H_{23}NO_2$	201	16.8	0.2	0.55
46	trans-4-Nonenedioic acid	$C_9H_{14}O_4$	186	21.58	0.35	0.23
47	Tetradecanedioic acid	$C_{14}H_{26}O_4$	258	23.4	0.45	0.15
	Total of identified compounds %				98.37	90.35

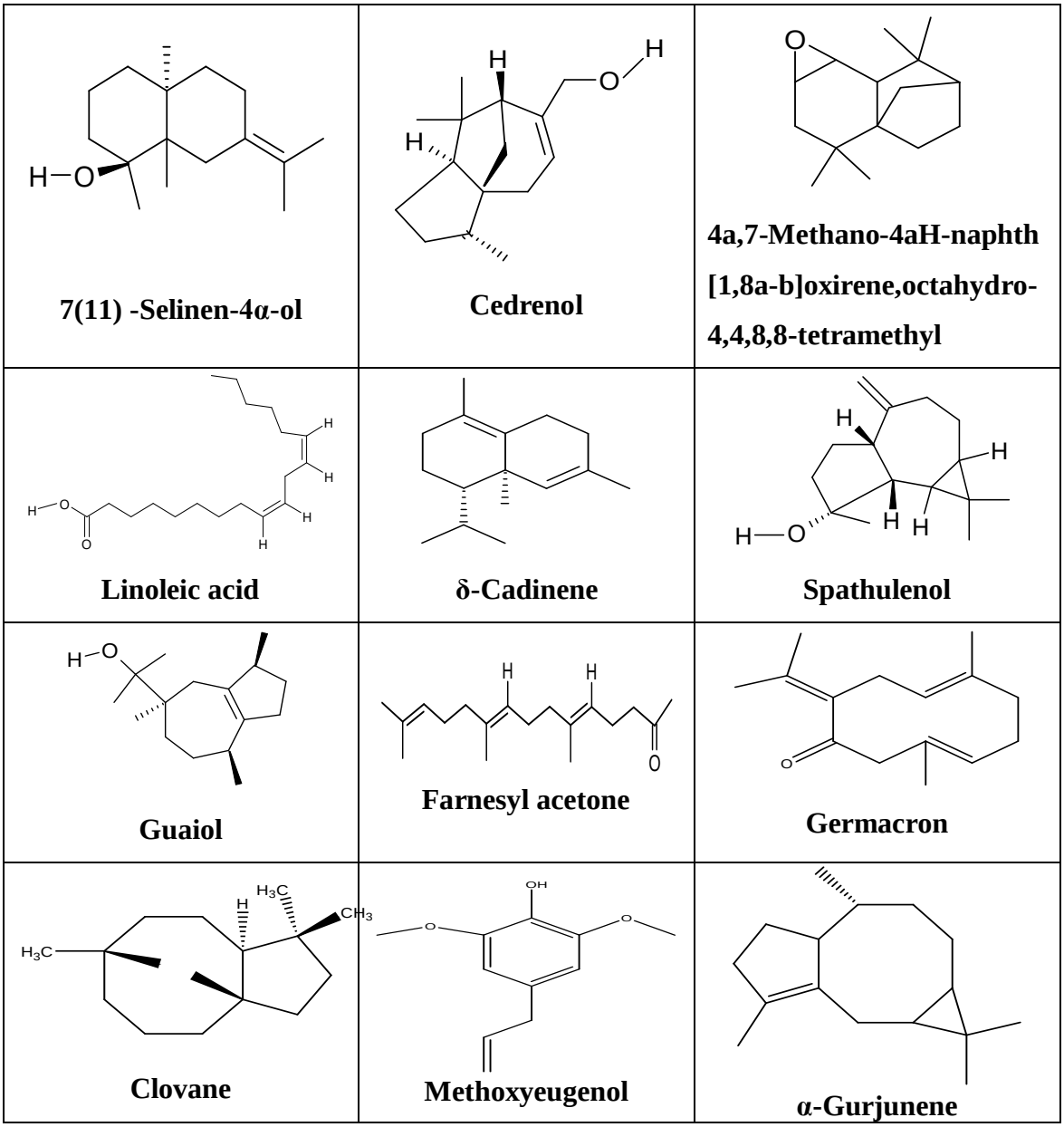

Fig (1): The structure of major identified compounds of essential oil of *L. camara* leaves and flower.

CONCLUSION

The present study showed the defatted methanolic extracts of *L. camara* and *C. pepo* and the ethyl acetate fractions derived from each defatted methanolic extract of both plants can be recommended as a new natural antioxidant agent. Also, there is a high correlation between the antioxidant activity and phenolic content of the two plants under investigation. The GC-MS analysis of *L. camara* (leaves and flowers) essential oils contains important bioactive compounds. Owing to the high antioxidant activity of the ethyl acetate and the butanolic fractions of the two plants under investigation, now the two fractions will be submitted to chromatographic isolation and identification of their chemical constituents.

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