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Original Article

Chemical Composition of Essential Oil, Anthocyanins and Fatty Acids of *Zinnia Pauciflora*

Hend E. Wahba[1]*, Hemaia M. Motawe[2], Abeer Y. Ibrahim[1]

[1]Research Medicinal and Aromatic Plants Dept., [2]Pharmacognosy Department, National Research Centre, Dooki, Cairo-12311, Egypt

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Abstract

The chemical composition of the herb volatile oil, anthocyanins in red flower and fatty acids in seeds of *Zinnia pauciflora*, family Asteraceae, were studied. Oxygenated compounds were the major volatile oil principle (89.8%) including iso-phytol (38.29%), hexandecanoic acid (12.41%) and spathulenol (9.35%). The major hydrocarbon was heneicosane (0.42%). Two anthocyanins were isolated from red flowers, cyaniding-3-gluco-(4-malonyl)-rhamnoside and cyanidine-3-("4-malonyl)-arabinoside. Seed total lipoidal matter represented 7.5% and the major fatty acids; Linoleic acid (34.98%), palmitic acid (17.27%) and oleic (13.33%).

Keywords: *Zinnia pauciflora*, anthocyanins, fatty acids, volatile constituents.

INTRODUCTION

Zinnia pauciflora is a member of family Asteraceae, an annual, perennial and sub shuraby plants [1] One of the reasons for the popularity of *Zinnia* is the cultivar diversity of their forms and colors of flowers; therefore, it is worthy to be of great commercial and aesthetic value although it is not famous like sunflowers or chrysanthemum. Zinnia is popular garden flowers and especially favored by butter flies and their flowers come in almost every shade except blue ones. One of them is *Zinnia pauciflora*, considered as one of the important ornamental plants in Egypt because of its successively and rapidly growing rate and also its use as cut flowers. Now it is used as a source of natural pigments, since the flavonoids are major compounds of *Zinnia*. Anthocyanins, flavons and flavonals were isolated from flowers and herb of *Zinnia* sp. [2, 3]. The volatile oil and fatty acids were obtained from *Zinnia* sp. Despite these reports information about the chemical composition of *Zinnia pauciflora* volatile oil, anthocyanins and fatty acids are lacking especially under the conditions of Egypt. Therefore, this study aimed to isolate and identify anthocyanins from red flowers, volatile constituents of herb and fatty acids of seeds. The major anthocyanins in red flowers of *Zinnia elegans* were pelargonidin 3, 5- Di-glucosides (30%) and cyanidin 3, 5-diglucosides (20%). Four anthocyanin components, pelargonidin 3-glucosides, pelargonidin 3.xylosylolucosides, cyanidin3-glucosides and cyanidin-3-xylosylglucosides were identified in *lycoris radiata* [4, 5] found that the main pigment of *Senecio cruentus* was 3-(6-malonyl-β-glucopyranoside)7-O-(6-O-trans caffeyL-B-D-glucopyranoside. Nakayama et al [6] isolated the cyanidin 3-0-(3, 6-0-malonyl-β-glucopyranoside from flowers of *chrysanthemum* plant. Los and Will et al. [7, 8] reported that *Prunus domestica* extract contained two major anthocyanins, cyanidin 3-0-0-glucoside and cyanidin 3-0-rutinoside. The analysis of flower extract of *Delonix regia*, anthocyanins content showed cyanidin 3-0-glucoside, cyanidin-3-0-rutinosid and pelargonidin 3-0 rutinoside [9] While anthocyanins from flower of *lycoris radiate* were cyaniding 3-diglucoside, cyanidin-3-sambubioside and cyanidin 3-glucoside, [10].

On the other hand [11] found that the chemical components of *Artemisia vulgaris* L, Asteraceae member, essential oil were germacrene (10.6-15.6 %), trans-thujone (20.2%), cis-thujone (12.9%) chrysanthenyl acetate (23.6%), 1, 8-cineole (16.7-17.6%). Mohammad-Bagher [12] reported that the essential oil composition of *Chrysanthemum balsamita* L., Asteraceae family, isolated from air dried aerial parts was carvon (42.53%), α-thujone (21.3%) and β-bisabolene (10.56%), Trans-p-menth-2,8-dienal (3.0%), β- thujone (2.21%) and β-cubebene (2.21%).[13] The major chemical

***Corresponding Author Address: Dr. Hend E. Wahba,** Research Medicinal and Aromatic Plants Department, National Research Centre.Dooki, Cairo, 12311, Egypt; E-mail: hend_wahba @yahoo.com*

composition of essential oils of *Stevia rebaudiana* Bertoni leaves were α-cadinol (2.98%), caryophllene oxide (1.23%), spathulenol (2.21%) and β-guaiene [0.32%]. The main constituents of oil in aerial parts of *Tagetes minute* were limonene, ocimene (monoterpenes), dihydrotagetone, taget one, tagetane, tagetenone and ocimenone [14] While the seeds oil, volatile oil composition of *Tagetes patula* was sesquiterpene (52.7%) and oxygenated sesquiterpenes (15.81%) followed by monoterpene hydrocarbons (2.6%).

The principle constituents of the volatile oil were E. caryophyllen (44.6%), caryophyllene oxide (14.8%), germacrene D (3.8%), Z.B. ocimene (3.8%) and limonene (3.7%) [15]. The main compounds of essential oil obtained from dried aerial parts of *Centaurea iberica* were germacreneD (20.3%) caryophylene oxide (10.7%) and B-caryophylene (10.5%) while *Centaurea solstitalis* subsp solstitalis were β- eudesmal (15.5%), bicycloger macrene (14.2%) and spathulene (11.3%) and *Centaurea virgate* were germacrene (21.4%) β-caryophyllen (16.5%) and carryophyllene oxide (9.5%) . The fatty acid of marigold seeds contains about 59% of an 18.3 conjugated trienic (Trans-8, trans-10, cis-12) acid and about 5% of g-hydroxy 18:2 (Trans-9, cis-11) acid, dimorphecolic acid [16]. Oxygenated fatty acid also reported from the seed oil of *Calendula afficinalis* was D-(+)-9-hydroxy-10, 12 octadecadienoic acid [17]. The main fatty acid accounted for oleic (41.75%) followed by myristic acid (21.51), then linoleic acid of *Zinnia elegans* [18] while the fatty acid of the green sunflower plant depends on its stage of maturity. The plant fatty acid profiles were characterized by four dominant fatty acid, palmitic acid (C16:0), Linoleic (C18:2), α-linolenic (C18:3) and stearidonic acid (C18:4) which ranged from 10.0-12.8, 16.4-21.8, 54.9-44.6 and 6.5-8.8% of the total fatty acid, respectively [19].On the other hand, Vosoughkia et al. [20] in experiment to evaluate of four safflowers (*Carthanus tinctorius* L.) genotypes. The oil content varied from 22.16 to 34.39%. The fatty acid were, linoleic acid (75.81-77.86%) and stearic (2.17-2.62%). The fatty acid composition in seeds of *Calendula officinalis* varied between 13.6-21.7g oil/100g seeds. The calandic and linoleic acids were the two dominant fatty acids in total lipid (51.4 to 57% and 28.5 to 31.9%) and triaylglceral (45.7 to 54.7 and 22.6 to 29.2%) fractions [21]. Also Vosoughkia et al. [22] identified the fatty acid profile of *Echinacea purpurea* seed oil as palmitic acid 16.6%, oleic acid 48% and linoleic acid 13.3%.

MATERIAL AND METHODS

Seeds of *Zinnia pauciflora* were brought from Combifleur Company, Netherland. The seeds were planted in nursery (1x1m) plots at the Experimental Farm of Faculty of Agriculture, Cairo University. The seedlings (40 days old) were transplanted to experimental plots at the end of March 2010, 60cm apart and 30 cm between plants. Dry herb and fresh red heads flower of *Zinnia pauciflora* were collected during July 2010.

For Paper chromatography (PC): Whatman 3MM and IMM sheets were from Whatman International Ltd. For thin layer chromatography TLC plates carried on microcrystalline cellulose LR (s.d.fine.chem.Ltd.) (MCC). All used solvents were technical grade (Aldrich, Germany).

Volatile compounds: Extraction and isolation of volatile fraction from *Zinnia pauciflora* fresh herb was according to [23].

Preparation of volatile fraction*:* The herb (150 g fresh) was subjected to hydrodistillation in a modified likens/and Nikerson apparatus which allowed the simultaneous extraction of volatile compounds in an organic solvent (pentane). The pentane layer was collected and analyzed by GC/MS.

Analysis of volatile compounds by GC/MS*:* The GC/MS analysis of the volatile constituents was carried out on gas chromatography directly coupled to mass spectrometer (Finningen SSQ 7000) applying the following conditions: Capillary column of DB-5 fused silica, 30 m length, 0.25 mm i.d. 0.25 µm thickness. Carrier gas helium at a rate of 10 ml/min. Temperature programming, 40-260°C increased at a rate of 3°C/ min, chart speed, 0.5 cm/min, ionization voltage, 70ev, detector, flame ionization detector. The identification of constituents was performed by comparing their retention times and mass fragmentation patterns with those of authentic and available references [24] .The quantitative determination was carried out based on peak area measurements.

Anthocyanins

Extraction of anthocyanins: Extraction of anthocyanins according to Yamaguchi *et al*., 1990, the fresh red flowers of *Z. pauciflora* (100 g) were soaked at room temperature in 5% HCOOH (500 ml), filtered through centered glass funnel, then concentrated under reduced pressure to give red soakage (about 20 ml).

Isolation and purification of anthocyanins: The soakage was fractionated on PC 3MM with n. butanol: actic acid: water (4: 1: 2) ascending technique then eluted and rechromatographed with

10% formic acid as solvent system. Seven spots were detected under UV and eluted by methanol 50% containing 5% formic acid. Two spots were eluted and spotted on microcrystalline cellulose plates and fractionated with different solvent system, n. butanol: formic acid: water (4:1:2), formic acid: water (1:9) and n.butanol: formic acid: water (6:1:2). The chromatoplates were examined under UV lamp of 254nm. Two compound were isolated and R_f value of each compound was recorded**, (Table, 2).**

Fatty acids

Extraction of lipoidal matter from the seeds: Seven grams of seeds powder were immediately extracted with petroleum ether (40-60°C) in a continuous extraction apparatus. The extract was evaporated under reduced pressure at 40°C. The total lipoidal matter obtained was about 0.4g.

Determination of fatty acids: Preparation of saponifiable and unsaponifiable lipoidal matter as well as fatty acid methyl esters were according to [25].The resulting fatty acid methyl esters were then analyzed by "Gas-Liquid Chromatography Technique". The qualitative identification of the fatty acids was achieved by comparing the retention time (R_t) of their peaks with those of authentics chromatographed under the same conditions. Gas liquid chromaograph/ PYE UNICAM PROGC instrument adapting the following conditions: Column: SP 2310, 55% Cyanoprophyl Phenyl Silicone Dimensions 1.5 x 4 mm. Temperature Programming: Intial Temp. 70°C Rate 5°C/ min, Final Time 25 min, Final Temp. 190°C, Injector Temp. 250°C, Detector Temp. 300, Gasses flow Rate: N_2 3 ml/ min, H_2 33 ml/ min, Air 330 ml/ min, Chart Speed 0.4cm/min. Carrier Pressure: N_2 0.6 atm, H_2 0.7atm Air 1.3 atm.

RESULTS AND DISCUSSION

Identification of the volatile constituents from herb of Zinnia pauciflora: The GC/ MS investigation of the volatile fraction from *Zinnia pauciflora* herb showed 17 peaks. Fourteen constituents were identified according to their mass spectra **(Table,1)** as; Iso phytol, , Hexadecanoic acid, Spathulenol, 7-ocabicyclo[4.1]heptan-1- methyl- 4- (2methyl oxytan). Phytol, Hencicosane, Pentacosane, hexanedioic dioctyl ester, 1,2,Benzennedi carboxylie acid, diiso-octyl ester, 6-methyl-2-Tridecanone , nonadecone, 2-7-heptadecym-ylory-tetrahydropyran, Palmitalde hyde diallyl-acetal, and 6,10,14-Trimethyl-2-pentadecanone. The majors of volatile principle were oxygenated compounds which constituted 89.87% of the total identified constituents. The major oxygenated hydrocarbons are iso-phytol (38.27%), hexandioic dioctyl ester (24.90%) and hexandecanoic acid (12.42%). The major non oxygenated compound is heneicosane (0.42%).

Table (1): GC/ MS analysis of the volatile constituents from herb of *Zinnia pauciflora.*

Peak No.	R_t	RR_t	Relative %	Base peak	M^+	Molecular Formula	Compound name
1	34.15	0.69	0.044	-	-	-	Unknown
2	37.14	0.76	9.348	43.91.55	220	$C_{15}H_{24}O$	Spathulenol
3	37.58	0.77	0.006	43.55.109	336	$C_{22}H_{40}O_2$	2-7-Heptadecymylory-tetrahydropyran
4	39.22	0.80	8.836	-	-	-	Unknown
5	42.12	0.85	4.850	43.108.93	168	$C_{10}M_{16}O_2$	7-Ocabicyclo[4.1]heptone- 1-methyl-4(2-methyl oxytan)
6	43.30	0.88	0.0037	43.58.59	268	$C_{18}H_{36}O$	6,10,14-Trimethyl-2- pentadecanone
7	45.01	0.92	0.0092	58.43.109	212	$C_{14}H_{28}O$	6-Methyl-2-Tridecanone
8	46.41	0.94	12.415	43.55.60	256	$C_{16}H_{32}O_2$	Hexandecanoic acid
9	49.15	1.00	38.27	71.43.56	296	$C_{20}H_{40}O$	Iso Phytol
10	49.40	1.01	0.004	84.43.55	322	$C_{22}H_{42}O$	Palmitadehyde diallyl- acetal
11	49.46	1.02	0.050	71	296	$C_{20}H_{40}O$	Phytol
12	54.00	1.09	0.001	-	--	--	Unknown
13	54.11	1.10	0.025	129.57.112	370	$C_{22}H_{42}O_4$	Hexanedioic, diocytl ester
14	55.40	1.13	0.007	57	268	$C_{19}H_{40}$	Nonadecone
15	56.42	1.15	0.018	148.166.54	390	$C_{24}H_{30}O_4$	1,2,Benzene di-carboxylie acid,di Iso-octyl ester
16	57.29	1.17	0.424	43.57.71	296	$C_{21}H_{44}$	Hencicosane
17	59.02	1.12	0.163		352	$C_{25}H_{52}$	Pentacosane

Table (2) Chromatographic and spectral properties of *Zinnia pauciflora* anthocyanins (A and B).

Compound	R_f x 100 in			In 01 % HCL-MeOH	
	AHW	BAW	BFW	X UV max (nm)	AL C13
Anthocyanin (A)	57	56	63	282	+
Anthocyanin (B)	45	58	68	282	+

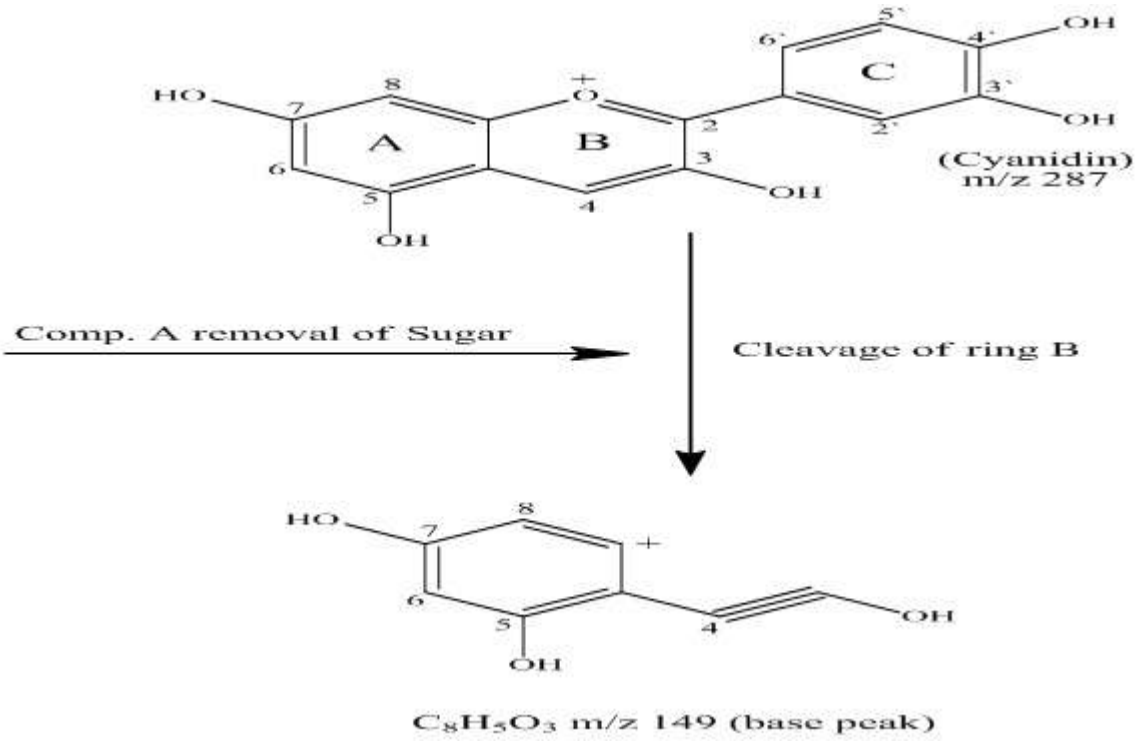
** TLC was carried on microcrystalline cellulose plates using AHW (HOAc-HC1-$H_2$0) (15: 3: 82), BAW (n.Bu0H- HOAc- $H_2$0) (6:1:2), BFW (n. Bu OH- HCOOH –$H_2$0) (4:1:2)

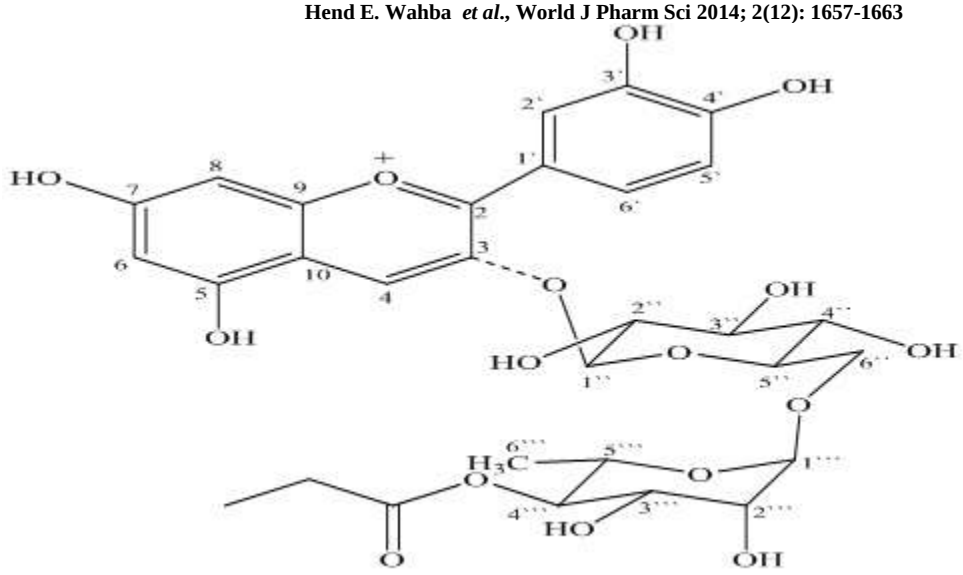
Identification of anthocyanins isolated from red flowers of *Zinnia pauciflora*

The isolated compounds A and B were subjected to spectral analysis; all results showed that both A and B are malonated anthocyanins.

Compound A

Compound A was completely soluble in methanol. It gave one single spot when chromatographed on microcrystallin cellulose plate and developed with different solvent systems, the R_f's values are tabulated in **Table (2).** The spectral data (UV, IR, MS and ^{1}H-NMR) of this compound revealed that it is related to malonated cyanidin anthocyanins. The MS fragments were to a high extent similar to those reported for a similar compound isolated from *Cichorias intybus* by Bridle *et al.* (1984), e.g. *m/z* 577.0647 for malonated cyanidine 3- gluco rhamnoside and m/z 449.1074 (cyanid-3-O--glucoside) Fig(1), and m/z 287.2401(cyanidin). Upon hydrolysis of this compound and investigation of its sugar moiety, glucose and rhamnose were detected and this was supported by the NMR. The NMR data did not show any acetyl radical signal and instead it showed a signal at 3.4 ppm. for malonyl radical which agreed with data obtain by [26]. The other signals at 3.29 ppm. for H-3", signal at 3.35 ppm. for CH_3, signals at 3.5-3.72 ppm. for H-5"', 3", 2" and 2"', signal at 3.90 ppm. for H-6", signal at 4.73 ppm. for H-1"', signal at 4.74 ppm. for H-4"', the signal at 4.80 ppm. for OH, signal at 5.44 ppm.for H-1", signal at 6.75 ppm. for H-6, signal at 6.93 ppm. for H-8, signal at 7.06 ppm. for H-5', signal at 8.02 ppm. for H-2', signal at 8.05ppm. for H-4 and signal at 8.26 ppm. for H-6'. The two main fragments at m/z 287.2401 and m/z 149.14875 (base peak) are explained. It is to be noted that m/z at 449.1076 indicated that the sugar attached to the cyanidin ring is glucose (Cyanidin-3- glucoside). The IR gave main bands at 3440 for (OH) group and 1650 for (C=0) group of malonyl.

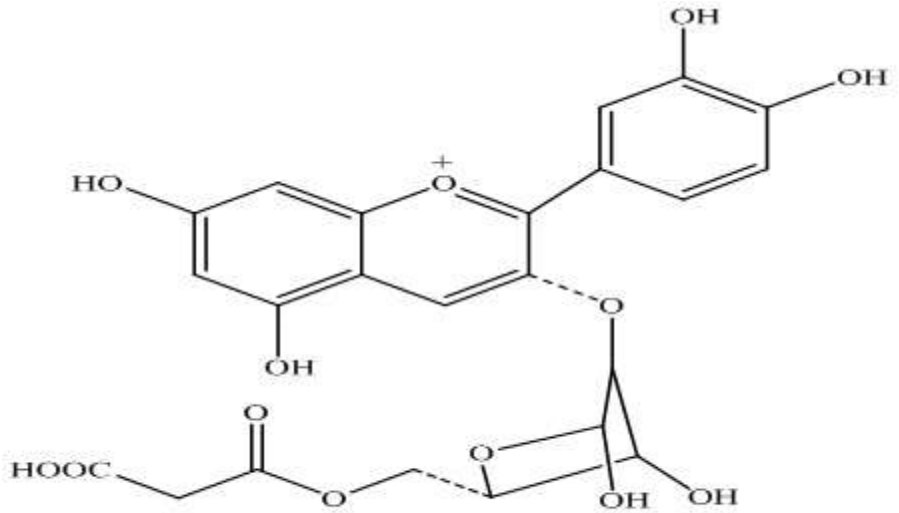




Compound A. Malonated cyanidine-3-glucorhamnoside

Compound B

Upon hydrolysis and investigation of compound B aglycone and sugar moiety in comparison with compound A. It contained arabinose only, while its aglycone was the same as compound A. Its IR and MS were similar to compound A. However, the NMR of its aglycone showed the same main signals as shown by compound A except those of the sugar signals. The malonyl signal was also clear in the NMR. The MS of compound B did not show fragments at m/z 449.1076 indicating absence of glucose but showed m/z 419.0971 for cyanidin-3-arabinoside. The two fragments at m/z 149.14875 (base peak) and m/z 287.2401 are explained by the same fragmentation path way as compound A. The IR showed characteristic bands at cm^{-1} 3442 for (OH) group and 1650 for (C=0) group of malonyl.



Compound B.Cyanidin-3-arabinoside malonate.

Analysis of fixed oil from seeds of Zinnia pauciflora: The fixed oil was extracted from the seeds of *Zinnia pauciflora* and the methyl ester of the fatty acids were prepared then sample (5µl) as well as authentic reference samples were subjected to GLC analysis. Identification of the fatty acids were carried out by matching the relative retention times of the detected peaks with those of pure available authentic samples. Quantitative estimation of each fatty acid relatively to total fatty acids was calculated based on area measurement.

GLC analysis of fatty acid of *Z. pauciflora* fixed oil: The total lipoidal matter of seeds of *Z. pauciflora* is 7.5%. The analysis of fatty acid methyl esters by GLC showed that the main fatty acids were linoleic (34.98%), oleic (13.33%) which represented 70.23% of total identified fatty acids and palmitic (17.27%) which represented (25.11%) of total identified fatty acids. The saturated fatty acids constituted (17.52%), the unsaturated fatty acids constituted (48.3%) and unknown compounds were about (30%) of the total fatty acids. The identified fatty acids agreed with those identified by[27] who found that analysis of fatty acid methyl esters of *Zinnia verticillata* showed that the percentage of oil (28.2%), linoleic acid (17.9%), palmitic acid (37.8%), stearic acid (8.2%), oleic acid (32.6%) of total fatty acids.

Table (3) GLC analysis of fatty acid methyl esters from seeds of *Z. pauciflora.*

Peak number	Components	Retention times (min)	Fatty acids (%)
1	Un known	0.91	15.320
2	Un known	1.48	9.533
3	Caproic (C6)	2.15	0.122
4	Caprylic (C8)	3.50	0.014
5	Lauric (C 12)	7.63	0.066
6	Myristic (C14)	11.07	0.048
7	Palmitic (C16)	13.02	17.270
8	Oleic (C18:1)	19.45	13.327
9	Linoleic (C18:2)	22.37	34.975
10	Un known	25.33	0.260
11	Un known	28.38	2.315
Total saturated fatty acids 17.52			
Total unsaturated fatty acids 48.30			
Total unknown compounds 27.428			
Total lipoidal matter 7.500			

CONCLUSION

The chemical composition of the herb volatile oil, anthocyanins in red flowers and fatty acids in seeds of *Zinnia pauciflora* were studied. Oxygenated compounds were the major volatile oil principle (89.8%) including iso-phytol (38.29%), (24.9%), hexandecanoic acid (12.41%) and spathulenol (9.35%). The major hydrocarbon was heneicosane (0.42%). Two anthocyanins were isolated from red flowers, cyaniding-3-gluco-(4-malonyl)-rhamnoside and cyanidine-3-("4-malonyl)-arabinoside. Seed total lipoidal matter represented 7.5% and the major fatty acids; Linoleic acid (34.98%), palmitic acid (17.27%) and oleic acid (13.33%).

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