IDENTIFICATION OF SOME VOLATILE COMPOUNDS EXTRACTED FROM CERTAIN MEDICAL PLANTS

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ABSTRACT

Stevia rebaudiana Bertoni, licorice (*Glycyrrhiza glabra L.*), carob (*Ceratonia siliqua L.*) and chicory (*Chichorum intybus L.*) are considered medicinal plants with many traditional uses such as sweetener plants. The volatile oils of these plants were isolated by steam distillation process. Identification of volatile compounds present in these volatile oils was assayed using Gas chromatography - Mass spectrometry (GC-MS). The volatile oil contents were about 0.24, 0.12, 0.08, and 0.27% of dried stevia leaves, licorice roots, carob pulp and chicory roots, respectively. The nerolidol, benzyl, δ.cadinene and caryophyllene were the major volatile compounds of stevia leaves, while methyl chavicol and benzaldehyde were the main ones of licorice. Carob pulp contained the hexanoic acid as the major component. The main constituents were benzoic acid, verapamil and verbenone in chicory roots. The volatile oils are very important for their activity as an anti-inflammatory drug, and also could be used as an anti-bacterial and flavouring agents in the field of food industry.

Keywords : volatile compounds, medical plants, sweetener plants, anti-inflammatory, antibacterial, flavouring agents

INTRODUCTION

Volatile oils, also called essential oils (EOs) are aromatic oily liquids obtained from plant materials by expression, fermentation, or extraction, but the method of steam distillation is most commonly used for commercial production of EOs (Van de Braak & Leijten, 1999). The greatest use of EOs in food is as flavourings and pharmaceuticals (for their functional properties). Some EOs or their components have been shown to exhibit antimicrobial (Nychas, 1995), insecticidal (Karpouhtsis *et al*., 1998), antitoxigenic (Juglal *et al.,* 2002), antiparasitic (Pessoa *et al.,* 2002), antiviral and antimycotic (Mari *et al*., 2003) properties.

Stevia rebaudiana Bertoni is an herbaceous perennial native to the highlands of Paraguay. It is especially known for the sweetening principles contained in the leaves, which have attracted the attention of both researchers and industrial producers because of their potential dietetic, alimentary and pharmaceutical interest (Jeppesen *et al*., 2000).

The leaves of stevia contain a complex mixture of labdone diterpenes, triterpenes, stigmasterol, tannins, volatile oils and eight sweet diterpene glycosides (Pasquel *et al.,* 2000). The dry herb yielded 0.12 – 0.16% essential oil. Chamazulene that isolated from the essential oil may be considered as a particular source of anti-inflammatory, antimitotic and antiasthmatic compound (Roman *et al*., 1990). The plant has also been used to treat different diseases such as diabetes, hypoglycemia, candidiasis, high blood pressure, skin abrasions and inhibiting growth and reproduction of bacteria-like plaque (Geuns, 2003).

Licorice (*Glycyrrhiza glabra L.*) is a flavourful herb that has been widely used in foods as a natural sweetener as well as a flavouring agent and can be added to foodstuffs in small quantities, with possible limitation of an active principle in the final product (Jiang *et al*., 2004). In USA, licorice is listed as known medicines in ancient history. It is a wonderful tonic herb for winter. This herb has long been valued as demulcent (soothing and coating agent) illnesses and immune, digestive tract, respiratory tract, and adrenal gland support (Luper, 1999). Furthermore, it has been used for bronchial catarrh, bronchitis, chronic gastritis, peptic ulcer, colic and primary adrenocortical insufficiency (Tamir *et al*., 2001). Also licorice root showed an antibacterial effect, it is used as an anti-inflammatory drug (Tanaka *et al.,* 2001) and as an antiallergic and antiasthmatic agent (Bielenberg, 2001).

There are many components in licorice but the most active one is glycyrrhizin that gives licorice its sweet taste. Other components are saponins (triterpenoid glycosides), flavonoids (including liquicritigetol) and isoflavonoids, bitter principle (glycyrmarin), volatile oil, chalcones, coumarins, amino acids, amines (choline, betaine, aspargine), oestrogenic substances (including beta-sitosterol), starch, sugars (glucose and sucrose), tannis, gums and wax (Michael, 2003).

Carob (*Ceratonia siliqua L.*) is a fruit from a dark evergreen tree that belongs to the family *leguminoseae.* Carob pods are nutritious due to their high sugar content, (Camacho *et al.,* 1996). The decoction of the pulp is antidiarrheal, gently helping to cleanse and relieve irritation within the gut (Aksit *et al.,* 1998). Other applications of pulps are in pharmaceutical products, sugar extraction (Marakis, 1992) and ethanol production (Roukas, 1994). Flavour and aroma are fundamental attributes of these products.

Chicory (*Cichorium intybus L.*) is a potential source of fructose for use as a sweetener in food, medicinal and energetic potential (Piet, 1996). Chicory root is known to be laxative, stomachic and diuretic. Its decoction is used as a tonic in fevers and for the treatment of jaundice, enlargement of spleen, gout and rheumatism (Hazra *et al.,* 2002). The same author added that, chicory contains the active component inulin. Herbalists recommend using this herb as an anti-inflammatory, choleretic, digestive tonic, laxative, mild diuretic and stomachic for anemia, liver disorders, kidney and gallstones, and urinary tract inflammations. It has been found also to significantly lower cholesterol and blood sugar levels. The sequiterpene lactones found in roasted root kill bacteria. Internally, it can be used for diabetes, dry coughs, abscesses, childbirth (second stage of labor), and abortion (tubers); bronchial infections with thick phlegm, chest pain and tightness; dry constipation, lung and breast tumor.

Therefore, the objectives of the present study were to identify, the volatile compounds in the aforementioned medical herbs.

MATERIALS AND METHODS

Materials

Stevia rebaudiana Bertoni leaves were obtained from El-Sabahia Station, Agricultural Research Center, Alexandria, Egypt.

Chicory (*Chichorum intybus L.*) was obtained from Agric. Res. Center Farm at Giza, Cairo, Egypt.

Licorice (*Glycyrrhiza glabra L.*) and carob (*Ceratonia Siliqua L.*) were obtained from local market in Alexandria, Egypt.

All plants used in the present study were dried in an air oven at 45±2ºC until reaching stable moisture content, then grinded and passed through 60-mesh sieve. Different sample powders were kept in polyethylene bags and stored at 4ºC until use.

Methods

Steam distillation process: One hundred grams from each of the dried plant materials were added to 1 liter of distilled water and subjected to steam distillation procedure for 2 hours. After distillation, the volatile oils were extracted from the condensed steam by hexane; the extract was concentrated in a rotary evaporator at room temperature. The extracted volatile oils were stored in airtight dark containers and kept in a refrigerator at 4 ºC until analysis (Martelli *et al.,* 1985).

Gas chromatography – Mass spectrometry (GC-MS): Identification and quantification of volatile compounds present in volatile oils were performed on the concentrated extracts of samples using a GC Hewlett - Packard 5890 series coupled with a MS-HP 5989B. One µl of volatile oil extract was injected into a glass capillary column PH 5; MS (5% diphenyl 95% dimethyl polysiloxane) (30 nm, 0.25 mm i.d., film thickness 0.25 um) using a splitless mode injector. The column inlet pressure was 5.3 Psi and helium was used as a carrier gas (0.8 ml/min). The oven temperature was programmed as follows: the initial temperature 100ºC for 6 min., then from 100ºC to 220ºC at 10ºC/min for 22min., and finally remaining at 220ºC for 40.5 min. The MS parameters were as follows, temperature of ion source, 125ºC, mass interval 50-550 m/e ionization energy, and

70ev (Cantalejo, 1997).The volatile compound was identified by library reference spectra (with a quality exceeding 85%) and quantified by integration of the mass spectrometer total ionization current by a real time computer .The concentration of volatile compounds were calculated by two methods , the first as percent of total area and the second as weight percent of the volatile oil.

RESULTS AND DISCUSSION

The volatile compounds of volatile oils obtained by steam distillation from Egyptian dried stevia leaves, dried licorice roots, dried carob pulp and dried chicory roots were analyzed using GC-MS. Chromatograms of volatile compounds of volatile oil samples are shown in Fig (1a, b, c, and d). Tables (1, 2, 3 and 4) show the identified volatile compounds: and their concentrations of the volatile oils. The yields of the volatile compounds were about 0.241, 0.116, 0.082 and 0.270% of the dried stevia leaves, licorice roots, carob pulp and chicory roots, respectively. Twenty-two volatile compounds were identified in stevia leaves extract. Among these compounds, nerolidol, benzyl alcohol, δ-cadinene, caryophyllene, caryophyllene oxide and α-humulene were the major components which represented 17.1, 13.8, 8.9, 6.9, 6.5 and 5.2%, respectively (Table 1). The obtained results are in agreement with Martelli *et al.* (1985) who reported that the major components in the essential oil of Brazil stevia were the sesquiterpenes caryophyllene oxide and nerolidol, where the monoterpenes were linalool, terpinen-4ol and terpineol. In contrast Tsanava *et al.* (1991) reported that caryophyllene oxide and spathulenol were the dominant, since they represent about 43% of stevia essential oil. These variances are mainly due to the different climatic and geographical conditions in which stevia plants are grown (Kinghorn, 2002 & Burt, 2004). The nerolidol, the dominant volatile compound of stevia, has strong antimicrobial activity and can be used for treatment or inhibition of microbial infections (Pauli & Schilcher, 2004). Also, benzyl alcohol is commonly used as an antibacterial agent in a variety of pharmaceutical formulations **(**Dasgupta & Humphrey ,1998**)**. From the above results and those mentioned in the literature, it could be noticed that stevia volatile oil extract contains rich proportion of aromatic aldehyde, monoterpenes and sesqui-terpenes, thus it can be used as a flavour enhancer in foods and food products (Buckenhuskers & Omran, 1997; Geuns, 2003).

Table (2) shows the volatile compounds of the volatile oil extracted from dried licorice powder. Licorice volatile compounds were found to contain nine compounds. Methyl chavicol (41.80%), linalool (16.87%), benzaldehyde (15.88%), 1, 8 cincole (7.47%), and furfuryl alcohol (6.99%) were the predominant components followed by hexanol (1.70%), α-terpineol (1.50%) and propionic acid (1.23%). These data agreed well with those of Tucker and Maciarello (1999) who found that the main constituents of volatile oil of licorice verbena leaf were methyl chavicol (56.46±4.29%) and 1, 8 cincole (12.62± 4.26%). On the other hand, Kameoka & Nakai (1987) identified that more than 80 components of licorice including anethole, benzaldehyde, butyrolactone, cumic alcohol, hexan-ol, furfuryl alcohol, indol, linalool, propionic acid, and α- terpineol. Katayoun *et al.* (2003) suggested the possibility of using a licorice extract as an effective natural antioxidant. Methyl chavicol (estragol), is an important constituent of many essential oils with widespread applications in folk medicine and aromatherapy and known to have potent local anesthetic activity (Cardoso *et at.,* 2004). Licorice root is used as a natural source of food flavouring, it is a wonderful medicine, real good seller in capsules and it has been used for bronchial catarrh, bronchitis, chronic gastritis, peptic ulcer, colic and primary adrenocortical insufficiency (Jiang *et al*., 2004)

Thirty volatile compounds of carob pulp were identified (Table 3). The major compounds were hexanoic acid (18.16%), 3-ethyl-5-methyl pyridine (10.69%), 2-octen-2one (6.27%) and octanoic acid (5.32%). The other identified volatile compounds occurred at concentrations of 5%. Macleod & Forcen (1992) found that, seven aliphatic acids represented an extraordinarily high level (77.5%) of the volatile components were isolated from carob beans oil. They mentioned that methyl propanoic acid (45%), hexanoic acid (19%) and 2-methyl butanoic acid (8%) are the major acids in volatile components of carob pulp.

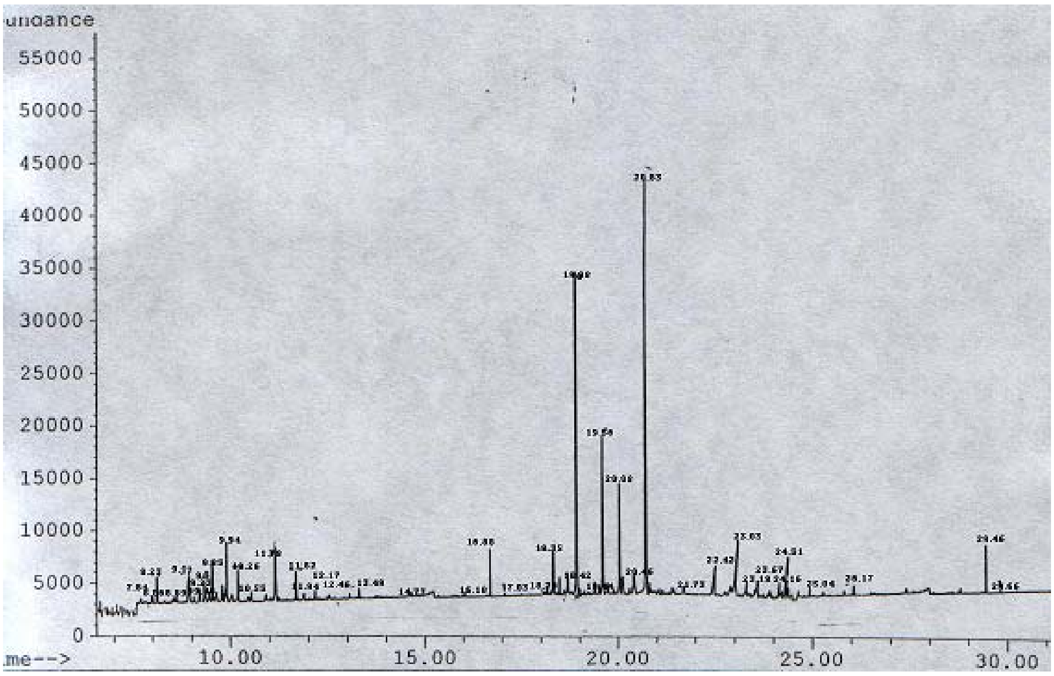

Fig (1.a): GC-MS Chromatogram of volatile compounds of stevia leaves

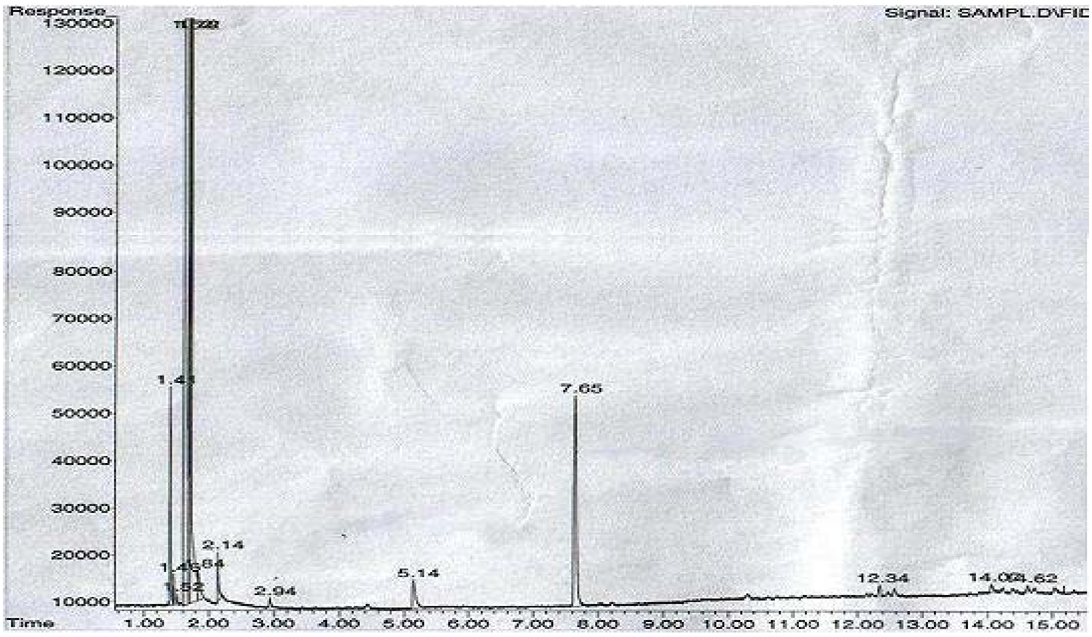

Fig (1.b): GC-MS Chromatogram of volatile compounds of licorice roots

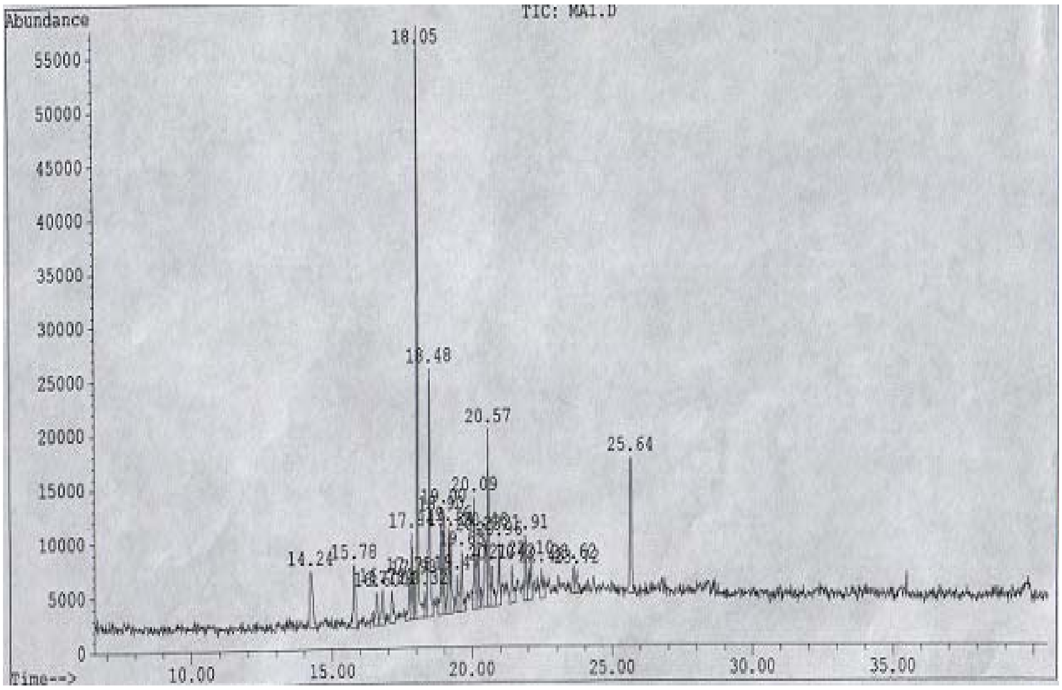

Fig (1.c): GC-MS Chromatogram of volatile compounds of carob pulp

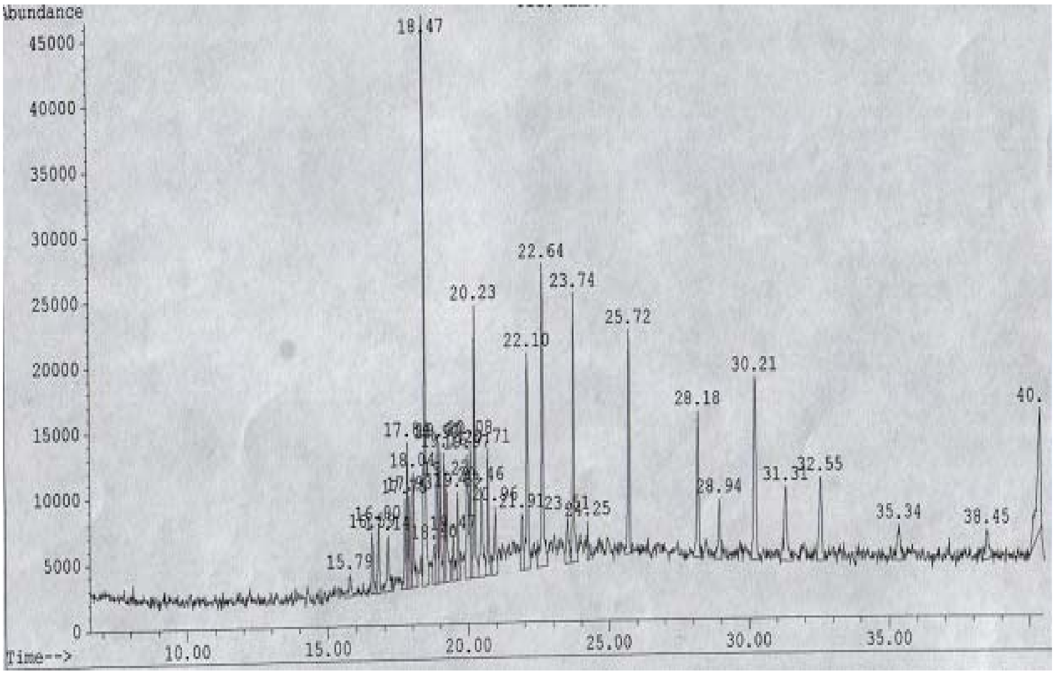

Fig (1.d): GC-MS Chromatogram of volatile compounds of chicory roots

Table 1: Volatile compounds identified by GC-MS in *Stevia rebaudiana*

Compounds	Retention time (min)	Concentration as	
		Area%	mg/100g
α - Pinene	7.84	0.9	2.16
n - Hexanal	8.06	1.1	2.64
Limonene	8.23	3.4	8.19
1,8 Cincole	9.17	2.2	5.30
γ- Terpinene	9.23	0.8	1.93
P – Cymene	9.46	1.9	4.57
3 – Octanol	9.73	0.9	2.17
1 – Octen – 3 ol	9.85	4.3	10.36
Linolool	9.94	3.9	9.39
Comphor	10.55	2.7	6.51
Caryophyllene	11.18	6.9	16.63
α – Humulene	11.82	5.2	12.53
Sesquilerpene ketones	16.88	4.1	9.88
α – Terpinol	18.35	3.3	7.95
Anethol	18.42	2.0	4.82
Benzyl alcohol	19.08	13.8	33.25
δ – Cadinene	19.56	8.9	21.44
Caryophyllene oxide	20.08	6.5	15.67
Nerolidol	20.83	17.1	41.21
Spothulenol	22.42	2.9	6.98
Eugenol	23.67	1.3	3.13
Carvacnol	24.51	2.9	6.98
7 unknown	9.53, 12.46, 13.44, 14.75, 20.46, 21.71, 26.17	3.0	7.20

Table 2: Volatile compounds identified by GC-MS in licorice

Compounds	Retention time (min)	Concentration as	
		Area %	mg/100g
1,8 cincole	2.14	7.47	12.84
Hexanol	1.52	1.70	1.97
Methyl chavicol	1.70	41.80	48.48
Linalool	1.41	16.87	19.56
Propionic acid	2.94	1.23	1.43
Furfuryl alcohol	5.14	6.99	8.11
Benzaldehyde	7.65	15.88	18.42
α – Terpineol	12.34	1.50	1.74
Butyrolactone	14.07	0.91	1.05
3 unknown	1.46, 1.84,14.62	5.65	6.54

Table 3: Volatile Compounds identified by GC-MS in carob

Compounds	Retention time (min)	Concentration as	
		Area%	mg/100g
Isobutyric acid	14.24	3.96	3.25
2- Methyl pentanoic acid	15.78	3.45	2.83
Caryophyllene	16.60	1.67	1.36
Phenyl acetaldehyde	16.80	1.71	1.40
4 – Terpineol	17.14	1.65	1.35
p – Vinyl guaiacol	17.74	1.80	1.47
Methyl benzoate	17.83	2.61	2.14
2 – Phenyl ethyl	17.92	1.14	0.93
Hexanoic acid	18.04	18.16	14.89
Octadecane	18.31	0.76	0.62
3 Ethyl–5 methyl pyridine	18.47	10.69	8.76
Benzoic acid	18.90	3.47	2.85
1 – butyl octyl benzene	19.01	2.87	2.35
Acetophenone	19.17	2.53	2.07
Methyl acetophenone	19.24	2.96	2.43
Hexacosane	19.47	1.75	1.44
3 Phenyl dodecane	19.63	2.45	2.0
Auraptene	20.08	3.65	2.99
1 butyl nonyl benzene	20.22	2.23	1.82
Methyl cyclopentenotone	20.45	3.68	3.02
Octanoic acid	20.58	5.32	4.36
Pentadecane	20.70	2.13	1.74
Ethyl cinnamete	20.97	2.42	1.98
Trimethyl gallic acid	21.42	2.18	1.79
Cumaldehyde	21.92	2.18	1.79
Heneicosane	22.09	2.28	1.87
Chavicol	22.49	1.95	1.59
Methyl chavicol (cymene)	23.61	0.97	.79
2 Methyl 2 ethyl propanoic acid	23.72	1.08	.89
2 Octen –2one	25.64	6.27	5.14

Cantalejo (1997) reported that acids, alcohols and aldehydes represent 91.4% of the total identified compounds in raw carob pulp. Volatile components of carob pulp could be used in foods as flavouring agents.

Data presented in Table (4) show that 37 volatile compounds were found in chicory roots but 31 only were identified as shown in Fig.(1-d).The volatile compounds extracted contains benzoic acid (11.16%) as a major constituent, followed by verapamil (6.67%), verbenone, 2,4,dimethyl 3-hexanone and iso-amyl salciylate.

Chicory roots contain relatively high amounts of 4 hydroxy phenylacetic, benzoic and 3,4 dihydroxycinnamic acids which contain 12 aromatic acids (Sannai *et al.,*1982). Baek & Cadwallader (1998) studied the volatile profiles of roasted chicory root by gas chromatography / mass spectrometry/olfactometry. Many pyrazines and n-furfuryl pyrroles were identified for the first time in roasted chicory. 2 ethyl 3-5-dimethylpyrazine 2,3-butanedione, 1-octen-3 one and 3-methylbutanol were the most intense aroma – active components found in roasted chicory.

Table 4: Volatile Compounds identified by GC-MS in chicory roots

Compounds	Retention time (min)	Concentration as	
		Area%	mg/100g
Benzyl benzote	16.58	1.33	3.59
4 – Terpineol	16.80	1.27	3.43
Methyl benzoate	17.85	2.12	5.72
2 Phenyl hexane	17.92	1.36	3.67
1 – Phenyl 2 ptapanone	18.04	2.27	6.13
Benzoic acid	18.47	11.16	30.13
Sesquibenihiol	18.79	1.07	2.89
1 – Pentyl heptyl benzene	18.90	2.13	5.75
1-Butanedione (butyl octyl benzene)	19.01	2.38	6.43
Acetophenone	19.17	1.71	4.62
1 – Propylnonyl benzene	19.24	2.06	5.56
Ethyl 5 – methyl pyrazine	19.63	1.48	3.99
5 – (Trimethylsilyl) methyl 2 phenyl	19.97	2.98	8.05
Dimethoxy benzaldehyde	20.08	2.28	6.15
2,4, Dioctyl phenol	20.24	4.06	10.96
1,3,3 Trimethylindole benzene	20.47	2.87	7.74
Eicosane	20.70	1.99	5.37
Auraptene	20.95	1.64	4.42
Cinnamyl cinnamate	21.92	1.27	3.43
Benzyl cinnamate	22.11	4.31	11.64
Verapamil	22.65	6.67	18.01
Thymol	23.50	1.39	3.75
Nonadecane (2,4 dimethyl 3hexanone)	23.73	5.29	14.28
2 Octen- 4one	25.71	4.80	12.96
Heneicosana	28.18	3.61	9.74
Dibutyl phenol	28.94	1.72	4.64
Iso amyl salicylate	30.21	5.22	14.09
Hexadecanoic acid	31.32	2.87	5.59
7-Trimethyl silyloxyl lavone	32.55	2.79	7.53
Dihydroxycinnamic acid	38.45	2.31	6.24
Verbenone	40.33	5.71	15.42
6 unknown	15.78, 17.13, 17.74, 19.47, 24.25, 31.32	6.68	18.04

In conclusion, the volatile oils of stevia leaves, licorice and chicory root have a characteristic flavour, due to the presence of many components with strong sensory properties at a low threshold, such as ethyl cinnamate and thus could be suitable for using as flavouring agent in food industry. These volatile oils can also be used as an antioxidant in foods, as well as anti-flammatory.

Finally, it is recommended that further studies are needed to measure the activity of such volatile compounds and their constituents in food products. In addition, safety studies have to be carried out as well.

DEDICATETION

Authors Mona, I. Massoud and Zeitoun, M.A. dedicate the present research paper to soul of Prof. Moussa, M.M. who suggested the problem of the work.

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التعرف على المركّباتِ الطيارة المستخلصة من بَعض النباتاتِ الطبيةِ

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أجريت هذه الدراسة على أربعة نباتات هى: الأستيفيا والعرقسوس والخروب والشيكوريا، والتي تتميز باحتوائها على مواد يعزى إليها التأثير الطبي، كما أن المحليات المستخلصة منها تستخدم في تحلية بعض المشروبات والأغذية الخاصة. لذا فإن هذا البحث يهدف إلى التعرف على المركبات الطيارة لتلك النباتات التي تتميز بفوائد عديدة طبية وغير طبية.

استخلصت المركبات الطيارة من النباتات موضع الدراسة بطريقة التقطير بالبخار وتم التعرف على تركيبها الكيماوي باستخدام طريقة كروماتوجرافيا الغاز/ مطياف الكتلة. تبين أن النسبة المئوية للزيوت الطيارة المستخلصة ٠,٢٤، ٠,١٢، ٠,٨، ٠,٢٧% لكل من نبات الأستيفيا والعرقسوس الخروب والشيكوريا المجففة على الترتيب.

وأوضحت الدراسة النتائج التالية:

أن مركبات نيروليدول ، كحول البنزيل ، δ كادنين وكاريوفللين هي أكثر المركبات السائدة في الزيوت الطيارة المستخلصة من أوراق نبات الاستيفيا المجففة، بينما يحتوي نبات العرقسوس على نسبة عالية من الميثايل كاثيول والبنزالدهيد واليناالول.

تميز نبات الخروب باحتوائه على نسبة عالية من حمض الهكسانويك.أما في جذور الشيكوريا فإن حمض البنزويك ومركبات فيرابامبل وفيرنيون كانت هي المركبات المستخلصة السائدة.

وتعتبر الزيوت الطيارة ذات فوائد دوائية ،كذلك فإنه يمكن استخدامها كمواد طبيعية مثبطة لنشاط البكتريا وكمواد مكسبه للنكهة في مجال الصناعات الغذائية.