Volatile compounds, antioxidants, and anticancer activities of Cape gooseberry fruit (*Physalis peruviana* L.): an *in-vitro* study

Manal M. Ramadan[a], Ahmed H. El-Ghorab[a,*], Kadry Z. Ghanem[b,#]

[a]Departments of Chemistry of Flavour and Aroma, [b]Food Science and Nutrition, National Research Centre, Giza, Egypt, *Department Chemistry, Faculty of Science, Aljouf University, Aljouf, KSA on loan from 2013-2015, #Clinical Nutrition Department, Faculty of Applied Medical Science, Jizan University, Jizan, KSA on loan from 2012-2015

Correspondence to Manal M. Ramadan, PhD, Chemistry of Flavour and Aroma Department, National Research Centre, Dokki, 12622 Giza, Egypt
Tel: +20 100 402 2497; fax: +20 23 337 0931; e-mail: manal.nrc88@yahoo.com

Received 05 May 2015
Accepted 25 November 2015

Journal of the Arab Society for Medical Research 2015, 10:56–64

Background/aim
Cape gooseberry is golden-colored spherical fruit commercially produced in Egypt. It is primarily used in folk medicine for treating some diseases. To identify the aroma compounds in Cape gooseberry and to evaluate its antioxidant activities as well as its anticancer (for colon and breast cancers) effects in human cell lines.

Materials and methods
The volatile compounds were identified using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Polyphenols (phenolics and flavonoids) were also determined. Antioxidant activity was determined by three different methods: 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays. Anticancer (for colon or breast cancer) activity was determined in cancer cell lines using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results
A total of 34 components of the essential oil were identified by GC and GC–MS. The volatile compounds were grouped in classes of substances, including 11 terpene compounds (six monoterpenoids and five sesquiterpene), 11 esters, five alcohols, two phenolic compounds, two aldehydes, two ketones, and one lactone. Terpenes (monoterpenes and sesquiterpenes) were the most abundant volatile constituents, accounting for the largest portion of the total volatiles (36.09%). The next most abundant compounds were esters, comprising 17.17% of the total volatile components identified. Phenolic compounds were the next most abundant compounds, comprising 16.04% of the total volatiles. Alcohols and aldehydes represented 6.37 and 1.88% of the total volatile compounds, respectively. Ketones and lactones are less abundant in the profile of volatile compounds in Cape gooseberry. Ethanol extract had higher phenolic and flavonoid contents than did hexane extract. As ethanol extract of Cape gooseberry achieved higher antioxidant activity than did hexane extract, it tested as an anticancer (for colon or breast cancer) agent. Cape gooseberry extract was more potent in inhibiting colon cell lines (IC_{50}: 142 μg/ml) compared with breast cell line (IC_{50}: 371 μg/ml).

Conclusion
Egyptian Cape gooseberry fruits may be suggested as a potential source of natural antioxidants and anticancer agents.

Keywords:
antioxidant, cancer, Egyptian Cape gooseberry, gas chromatography–mass spectrometry, polyphenols

J Arab Soc Med Res 10:56–64

1687-4293

Introduction

Functional foods represent an emerging market of growing economic importance. International markets exist for many exotic fruits, and recently the processing of tropical fruits started in many countries [1]. In 2005, there were more than 1.8 million acres of berry crops worldwide including 966 acres of gooseberries [2].

Cape gooseberries are annuals or short-lived perennials, and are flavor and appearance, though the taste (sweet and sour) is much richer with a hint of tropical luxuriance. The plant is fairly adaptable to wide variety of soils and good crops are obtained on poor sandy ground [1,3].

Cape gooseberry (*Physalis peruviana* Linn., Solanaceae) has been grown in Egypt, South Africa, India, New Zealand, Australia, and Great Britain [4,5]. Cape gooseberry (*P. peruviana* L.) is a cherry-sized, yellow-fleshed intriguing berry, which was originally cultivated in the Andes. The round orange fruit is loosely enclosed in a papery husk, which provides a natural wrapper for storing

the fruit, as long as it is kept dry. Cape gooseberry is used in folk medicine for treating diseases such as malaria, asthma, hepatitis, dermatitis, diuretic, and rheumatism [6,7].

Many medicinal properties have been attributed to Cape gooseberry, including antiasthmatic, antiseptic, and strengthener for the optic nerve, and it is used in the treatment of throat affections and elimination of intestinal parasites, amoebas as well as albumin from kidneys. It has an antiulcer activity and it is effective in reducing cholesterol level [8,9]. Berries have been shown to provide significant health benefits because of their high antioxidant content [2].

In addition to having a future as a fresh fruit, the fruit can be consumed in many ways as an ingredient in salads, cooked dishes, dessert, jam, natural snack, and preservers. Its extract can also be used for preparing a health drink [5]. This will be important as an indication of the potentially nutraceutical and economical utility of Cape gooseberry as a new source of bioactive phytochemicals and functional foods. The extracts can provide a cheap and sustainable method toward disease reduction and can eventually improve the quality of life of the rural and periurban poor in developing countries [10].

Aroma and flavor are among the most important attributes and quality criteria that affect the consumption of fruits, and both qualitative and quantitative information is desired for characterizing aroma-producing compounds [11]. However, many synthetic antioxidants used in foods, such as butylated hydroxyanisole and butylated hydroxytoluene, may accumulate in the body, resulting in liver damage and carcinogenesis [12]. For this reason, more attention has been paid to natural nontoxic antioxidants in an effort to protect the human body from free radicals and retard the progress of many chronic diseases, especially cancer. Recently, it was found that many natural extracts from plant sources posses high antioxidant activity and play an excellent role as free radical scavengers in human body [13–15].

Natural extracts from different plants growing in Egypt posses high antioxidant activity and high content of phytochemicals [16–18]. According to our knowledge, very little information exists regarding the chemical composition of volatiles and antioxidant activity as well as bioactive effects of Egyptian Cape gooseberry extracts. Thus, this study has four main objectives:

(1) To identify the volatile compounds of Egyptian Cape gooseberry fruits by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS);
(2) To determine phenolic and flavonoid content of the Cape gooseberry fruit extracts;
(3) To evaluate the antioxidant capacity of its extracts by three different methods; and
(4) To evaluate the anticancer (breast and colon) cell lines of its extract.

Methods

Plant

Egyptian Cape gooseberries (*P. peruviana* L.), widely cultivated in Nil Valley, were obtained from a local market of Cairo (Egypt). Completely healthy fruits were selected and analyzed as a whole.

Extraction of volatile components

Cape gooseberry fruits were cut to small pieces with a knife and blended for 3 min with a blender (Moulinex, France). Tissues of Cape gooseberry fruits were rapidly juiced and the volatiles were isolated using a dynamic headspace system. The sample was purged for 3 h with nitrogen gas at a flow rate 100 ml/min. The headspace volatiles were swept into cold traps containing diethyl ether and held at −10°C. The volatile extracts were dried over anhydrous sodium sulfate for 1 h, and then reduced to 1 ml by using rotary evaporator (Heidolph, Germany) [19].

Identification of volatile compounds

Gas chromatography analysis

About 2 µl of each pure volatile oil was used. GC analysis was performed by using Hewlett-Packard model 5890 (Hewlett-Packard, Perkin elmer Co., USA) equipped with a flame ionization detector. A fused silica capillary column DB-5 (Zebron Co., USA) (60 m × 0.32 mm, internal diameter) was used. The oven temperature was maintained initially at 50°C for 5 min, and then programmed from 50 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas, at a flow rate of 1.1 ml/min. The injector and detector temperatures were 220 and 250°C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C7–C21; Sigma-Aldrich Co.) as references [20].

Gas chromatographic–mass spectrometric analysis

The analysis was carried out by using a coupled GC Hewlett-Packard model 5890/MS Hewlett-Packard MS 5970 (Hewlett-Packard). The ionization voltage was 70 eV, and the mass range m/z was 39–400 a.m.u. The isolated peaks were identified by matching

with data from the library of mass spectra (National Institute of Standard and Technology), and compared with those of authentic compounds and published data. The quantitative determination was carried out on the basis of peak area integration. Identification of the GC components was also confirmed with the help of National Institute of Standard and Technology mass spectra library data, as well as on comparison of their retention indices with those of authentic compounds [20].

Preparation of extracts of Cape gooseberry fruits

Nonvolatile (phenolics and flavonoids) compounds were extracted from Cape gooseberry fruits with a modification, as reported by Rajeswari *et al*. [21]. Two different solvents (hexane and ethanol) were used for preparation of two different extracts. Briefly, 100 g fresh fruits were cut into small pieces and extracted using 500 ml hexane. In another flask, the same amount (100 g) of fresh fruits were extracted with the same volume (500 ml) of 95% ethanol. Two flasks were shaken every hour for the first 6 h and then were kept aside, and again shaken after 24 h. The solvent layer was separated from the solid residue by centrifuging at 2000*g* for 10 min. The clear supernatant was transferred to a clean rounded flask and evaporated using a vacuum evaporator at less than 50°C. The extract concentrated to 2 ml was stored at −20°C until samples were subjected to the following:

Determination of phenolic and flavonoid content

The phenolic content was determined according to the Folin–Ciocalteu procedure [22]. It was determined by means of a calibration curve prepared with gallic acid, and expressed as mg of gallic acid equivalent/ml of sample. The total flavonoid content was determined as reported by Thaipong *et al*. [22], and was expressed as mg of catechin equivalent/ml of sample.

Determination of antioxidant activity

Determination of radical 2,2´-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH assay was carried out as reported by Thaipong *et al*. [23]. The antioxidant activity was determined by means of a calibration curve prepared with ascorbic acid, and expressed as mg of ascorbic acid equivalent/ml of sample.

2,2-Azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assay: For ABTS assay, the procedure followed the method described by Arnao *et al.* [24]. Results were expressed in mmol/l Trolox equivalents (TE)/ml extract. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was carried out according to Benzie and Strain [25]. Results were expressed in mmol/l TE/ml extract. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

Anticancer activity: cell cultures and treatments

Human breast cancer cell line (MCF-7) and colon cancer cell line (Caco-2) were obtained from the American Type Culture Collection (Rockville, Maryland, USA). Cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% nonessential amino acid solution, and 1% penicillin–streptomycin solution (10 000 U of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2, 95% air at 35°C. The passage number range for both cell lines was maintained between 20 and 25. The cells were cultured in 75 cm^2 cell culture flasks. For experimental purposes, cells were cultured in 96-well plates (0.2 ml of cell solution/well). The optimum cell concentration as determined by the growth profile of the cell line was 2×10^5 cells/ml (cells were allowed to attach for 24 h before treatment with tested extracts). The stock solution was filtered with Minisart Filters Merck (Darmstadt, Germany) (0.22 μm). Working two-fold serially diluted test material (1, 2, 5, 10, 20, 40, 80, 162, 325, 750, 1500, and 3000 μg/ml) were prepared. Cell monolayers were washed with PBS and the additional serially diluted materials were dispensed to the precultured plates for the determination of test material's toxicity [26].

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay: The MTT assay is based on the protocol described for the first time by Mossmann [27]. The assay was optimized for the cell lines used in the experiments. Briefly, for the purposes of the experiments at the end of the incubation time, cells were incubated for 4 h with 0.8 mg/ml of MTT dissolved in serum-free medium (MEM or DMEM for MCF-7 and Caco-2 cells, respectively). Washing with PBS (1 ml) was followed by the addition of DMSO (1 ml), and gentle shaking for 10 min so that complete dissolution was achieved. Aliquots (200 μl) of the resulting solutions were transferred in 96-well plates, and absorbance was recorded at 560 nm using the microplate spectrophotometer system (SpectraMax 190; Molecular Devices, Germany). Results were analyzed with the SoftMax Pro Software, Germany

(version 2.2.1) and were presented as percentage of the control value. The relation between surviving fraction and extract concentration was plotted to get the survival curve for the cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was also calculated [27].

Statistical analysis

The experiments were carried out at least three times, and the results were presented as mean ± SD. Statistical differences were analyzed by using the one way ANOVA test.

Results

Gas chromatography and gas chromatographic–mass spectrometric analysis

A total of 34 components were identified by GC and GC–MS analysis. The volatile compounds were grouped in classes of substances, including 11 terpene compounds (six monoterpenes and five sesquiterpenes), 11 esters, five alcohols, two phenolic compounds, two aldehydes, two ketones, and one lactone, as shown in Table 1. Terpenes (monoterpene: 20.81% and sesquiterpene: 15.28%) were the most abundant volatile constituents, accounting for the largest portion of the

Table 1 Chemical composition of volatiles of Egyptian Cape gooseberry fruits

Type of compounds	Identified compounds	Area%	R_t	KI	Method of identification
Monoterpenes	Methyl butene	1.25	5.55	528	MS, KI
	α-Pinene	1.26	14.57	938	MS, KI, and St
	Verbenene	0.93	15.97	972	MS, KI
	1,8-Cineol	1.74	18.88	1038	MS, KI, and St
	Verbenone	3.5	26.21	1199	MS, KI
	Citronellyl acetate	12.13	33	1355	MS, KI
Total		20.81			
Sesquiterpenes	δ-Muurolene	7.71	32.06	1333	MS, KI
	α-Cubebene	1.06	32.44	1344	MS, KI, and St
	Cyclosativene	2.23	32.85	1351	MS, KI
	Cedrenol	1.26	42.76	1604	MS, KI
	β-Bisabolol	3.02	45.08	1665	MS, KI, and St
Total		15.28			
Total terpene compounds		36.09			
Esters	Methyl butanoate	8.95	8.11	727	MS, KI
	Propyl hexanoate	3.73	22.13	1110	MS, KI
	Benzyl acetate	0.77	24.27	1157	MS, KI, and St
	Ethyl benzoate	2.51	24.65	1165	MS, KI
	Ethyl octanoate	1.26	25.70	1188	MS, KI
	Ethyl hydroxyl hexanoate	0.13	31.69	1326	MS, KI
	Neryl acetate	2.83	33.33	1363	MS, KI
	Allyl caproate	0.71	38.14	1480	MS, KI
	Hexadecanoic acid ester	1.05	44.03	1636	MS, KI
	Cedr-8-en-9-alpha-ol acetate	0.93	47.39	1740	MS, KI
	Phenyl ethyl benzoate	2.97	51.91	1865	MS, KI
Total		17.17			
Phenols	4-Propyl guaiacol	1.28	49.97	1806	MS, KI
	isoeugenol	14.76	57.53	2042	MS, KI, and St
Total		16.04			
Alcohols	Butanol-2-methyl	0.95	5.65	567	MS, KI, and St
	2-Phenyl ethyl alcohol	1.5	22.45	1117	MS, KI, St
	Nonanol	1.0	22.60	1120	MS, KI
	Homofuraneol	1.5	24.13	1154	MS, KI
	Dihydrocarveol	1.42	42.44	1592	MS, KI
Total		6.37			
Aldehyde	p-Anisaldehyde	0.72	29.19	1266	MS, KI
	2-Undecenal	1.16	32.76	1349	MS, KI
Total		1.88			
Ketones	3,5-Octadienone	0.83	21.69	1096	MS, KI
	Trimethyl phenyl butenone	0.12	47.77	1751	MS, KI
Total		0.95			
Lactones	γ-Undecalactone	0.76	43.03	1608	MS, KI

KI, Kovats index; MS, mass spectrum; R_t, retention time; St, standard compounds.

total volatiles (36.09%). Citronellyl acetate, followed by δ-muurolene, verbenone, and β-bisabolol were the terpenes found in highest concentration. The next most abundant compounds were esters, comprising 17.17% of the total volatile components identified. Methyl butanoate, followed by propyl hexanoate, phenyl ethyl benzoate, neryl acetate, and ethyl benzoate were the esters found in the highest concentration. Phenolic compounds were the next most abundant compounds, comprising 16.04% of the total volatile components determined. Isoeugenol represented 14.76%, whereas 4-propyl guaiacol represented 1.28%. Alcohols represented 6.37%, whereas aldehydes represent 1.88% of the total volatile compounds. 2-Undecenal was the predominant aldehyde. 3,5-Octadienone was the major constituent among the ketones, which accounted for the 0.95% of the identified volatile constituents. Lactones comprised small part (0.76%) of the total volatiles identified.

Total phenolic and flavonoid content

The amounts of total phenols and flavonoids in the hexane and ethanol extracts of the Cape gooseberry fruits are shown in Table 2. The total phenolic contents of hexane and ethanol extracts of Cape gooseberries fruits were 30.0 ± 1.1 and 89.6 ± 2.1 mg gallic acid equivalent/ml extract. The total flavonoid contents of hexane and ethanol extracts of Cape gooseberries fruits were 21.3 ± 1.1 and 77.1 ± 3.1 mg catechin equivalent/ml extract.

Antioxidant activity

This study evaluated the role of gooseberry fruit extracts as antioxidants *in vitro* by using three different methods: ABTS, ferric reducing antioxidant power (FRAP), and DPPH (Table 3). Data showed that ethanol extract exhibits stronger antioxidant activity than does hexane extract. Means of the antioxidant activity levels in ethanol extract were 1.785 ± 0.02 and 1.922 ± 0.03 mmol TE/ml extract as determined by ABTS and FRAB assays, and 2.016 ± 0.03 mg ascorbic acid equivalent/ml extract by DPPH assay, whereas means of the antioxidant activity in hexane extract were 0.611 ± 0.01 and 0.713 ± 0.02 mmol TE/ml extract by ABTS and FRAB assays and 0.903 ± 0.01 mg ascorbic acid equivalent/ml extract by DPPH assay, as shown in Table 3. Ethanol extract achieved the highest antioxidant activity as well as high total flavonoid and total phenolic content, and thus tested its activity as anticancer.

Anticancer activity of Cape gooseberry fruits

The Cape gooseberry ethanol extract showed growth inhibition as anticancer. The in-vitro cytotoxicity was performed against two different human cancer cell lines, namely breast (MCF-7) and colon (Caco-2). Comparison of the mean MCF-7 and Caco-2 cell viabilities analyzed using the MTT method indicated a significant difference between them ($P < 0.05$). Increasing the dose significantly decreased the cell viability in both types of cancer cell lines. In the case of colon Caco-2 cell line, Cape gooseberry fruit extract showed maximum activity (Fig. 1). Cape gooseberry fruit extract was more potent in inhibiting colon cell lines (IC_{50}: 142 µg/ml) compared with breast cell lines (IC_{50}: 371 µg/ml) (Fig. 2).

Discussion

Total Cape gooseberry aroma is the result of the presence of different compounds such as alcohols,

Table 2 Phenolics and flavonoids content of ethanol and hexane extracts of Egyptian Cape gooseberry fruits

Cape gooseberry extracts	Phenolics (mg/ml)[a]	Flavonoids (mg/ml)[b]
Ethanol extract	89.6 ± 2.1[c]	77.1 ± 3.1[c]
Hexane extract	30.0 ± 1.1[d]	21.3 ± 1.1[d]

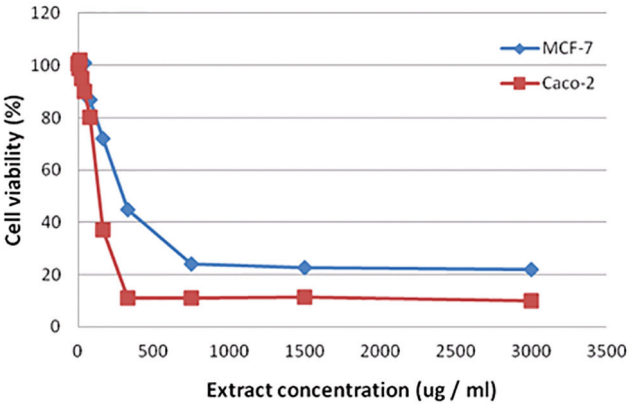
All data are presented as mean ± SD; [a]Results expressed as mg of gallic acid equivalent/ml extract; [b]Results expressed as mg of catechin equivalent/ml extract; Values in the same column with the different superscripts are significant at $P < 0.05$.

Table 3 Antioxidant activity of ethanol and hexane extracts of Egyptian Cape gooseberry fruits by ABTS, FRAB and DPPH assays

Cape gooseberry extracts	DPPH (mg AAE/ml)	FRAB (mmol/l TE/ml)	ABTS (mmol/l TE/ml)
Ethanol extract	2.016 ± 0.03[a]	1.922 ± 0.03[a]	1.785 ± 0.02[a]
Hexane extract	0.903 ± 0.01[b]	0.713 ± 0.02[b]	0.611 ± 0.01[b]

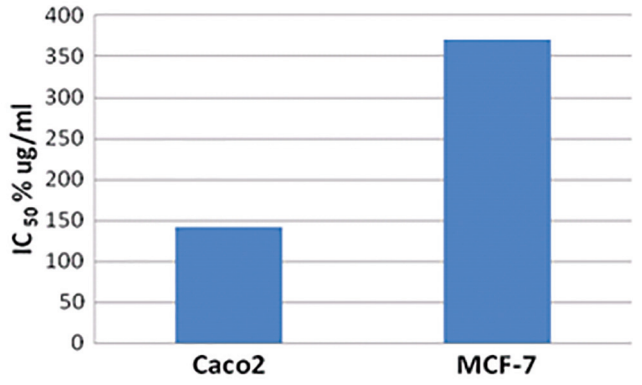
All data of inhibition activity are presented as mean ± SD; AAE, ascorbic acid equivalent; ABTS, 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid); DPPH, 2,2´-diphenyl-1-picrylhydrazyl; TE, Trolox equivalent; Values in the same column with the different superscripts are significant at $P < 0.05$.

Figure 1



Cytotoxic effect of Cape gooseberry fruit extract against MCF-7 breast and Caco-2 colon cancer cell lines.

Figure 2



IC_{50} (μg/ml) of Cape gooseberry fruit extract against MCF-7 breast and Caco-2 colon cancer cell lines.

esters, terpenes, aldehydes, ketones, and lactones. Among them, esters are the most important group because they are responsible for fruity and fresh flavor [28]. Our results are in agreement with a study conducted by Yilmatekin [28], which demonstrated the presence of esters, aldehyde, alcohols, terpenes, ketones, and lactones in Turkish Cape gooseberry. Terpenes were the most abundant volatile constituents derived by repetitive fusion of branched five carbon units based on isopentane skeleton. Many of them were volatile, such as monoterpenes (C_{10}), and sesquiterpenes (C_{15}).

Terpenes are derived either from mevalonate pathway, which is active in cytosol and starts from acetyl-CoA, or from methylerythritol-4-phosphate pathway, which is active in the plastids and starts from pyruvate and glyceraldehyde-3-phosphate [29]. In contrast, the biosynthesis of some terpene-derived compounds can be explained by catabolic pathways in fruits. These are primarily oxidative degradation products of the carotenoids. Carotenoid oxidation occurs when the plant tissue is damaged or during ripening [30]. Terpenes and their derivatives have been identified at varying levels in most of the soft fruits [31] and they are responsible for the varietal character of the fruits being present, at least, in part, as glycosides [32].

They were reported as volatile components responsible for a wide spectrum of aromas (woody, piney, turpentine-like, herbaceous, and terpy), mostly perceived as very pleasant [32,33]. Esters contribute to the aroma of nearly all fruits and many other foods. Some are also responsible for the smell of a particular flower; however, many of these esters possess a nonspecific fruity odor. As the number of carbon atoms increases, the odor changes to fatty soapy and even metallic. The straight-chain ester constituents are believed to be synthesized through β-oxidation of fatty acid, which may be then reduced to the corresponding alcohols before transesterification [34].

Alcohol acyltransferases are responsible for the transfer of alcohol to acyl-CoA, resulting in the synthesis of a wide range of esters [32,35,36]. Aldehydes are common in fruit flavors and are believed to play an important role in many fruits [37]. Fatty acids and amino acids are precursors of a great number of volatile aldehydes. Linoleic and linolenic acids in fruits and vegetables are subjected to oxidative degradation by lipoxygenase alone or in combination with a hydroperoxide lyase. The oxidative cleavage yields oxoacids, aldehydes, and allyl alcohols [32,38]. Ketones are less abundant in the profile of volatile compounds in Cape gooseberry. The ketones can be formed by condensation of activated fatty acids [39].

Lactones are produced in a very low amount by catabolic processes and originate from their corresponding hydroxyl carboxylic acids (4-hydroxy carboxylic acid or 5-hydroxy carboxylic acid) [40]. These compounds, particularly γ-lactones, are important in terms of their contribution to the aroma and, in general, present fruity odor descriptors [33]. The odor of these lactones depends on the chemical structure, functional groups, and the length of side chains, and due to their low odor threshold, they have a high flavor value in fruits [40]. Despite its importance, the literature about the flavor compounds of volatiles of Cape gooseberry (*P. peruviana* L.) is scarce.

The results of the present study indicated that the ethanol extract has higher total phenolics content than does hexane extract ($P < 0.05$). In addition, ethanol extract has higher total flavonoid compounds than does hexane extract. According to these results, there is a relationship between phenolic and flavonoid contents and radical scavenging activity. The differences in the amount of polyphenols may be due to varied efficiency of the solvents to dissolve endogenous compounds. Phenolic compounds, biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups [41]. Various bioactive compounds (flavonoids and phenolics) are reported to be present in *P. peruviana* [42]. Some of these compounds have a strong antioxidant property and prevent peroxidation [43].

It is very important to point out that there is a positive relationship between antioxidant activity potential and the amount of phenolic compounds of the extracts. From the phenol antioxidant index, a combined

measure of the quality and quantity of antioxidants in vegetables has been obtained [44]. Data showed that antioxidant activity of ethanol extract of gooseberry fruits increased ($P < 0.05$) 2.921, 2.69, and 2.23 times that of hexane extract by ABTS, FRAB, and DPPH methods, respectively. These results are in agreement with studies conducted by Matkowski and Piotrowska [45] and Li *et al*. [46].

Narváez-Cuenca *et al*. [47] indicated that gooseberry (*P. peruviana* L.) has a high antioxidant activity. The high antioxidant capacity of the fruits is probably due to their richness in oxygenated monoterpene compounds. The stronger antioxidant activity exhibited by Cape gooseberries, in both the DPPH test and ABTS assay, confirms results showing that some of the oxygenated monoterpenes are mostly responsible for protective effects [48]. The antioxidant activity of volatile compounds was variable; this variability is mainly related to their molecular composition.

Egyptian Cape gooseberry fruit content bisabolol compound is a monocyclic sesquiterpene alcohol. Baraga *et al*. [49] showed that bisabolol has an antioxidant/anti-inflammatory activity. The antioxidants are an increasingly important ingredient in food processing. The most widely used synthetic antioxidants in food (butylated hydroxytoluene, butylated hydroxyanisole) are very effective in their role as antioxidants. However, their use in food products has been failing off because of their instability, as well as because of a suspected action as promoters of carcinogenesis [50]. Consequently, there has been considerable interest in the use of antioxidant compounds from natural sources to exhibit different biological properties [51]. They protect the human body from free radicals and retard the progress of many chronic diseases, especially cancer. In the present study, ethanol extract of Cape gooseberry fruit achieved higher antioxidant activity than did hexane extract, and thus tested as having anticancer activity.

Up to this day, in spite of the great nutritional value of Cape gooseberry fruit, studies about its effect on colon or breast cancer are scarce. According to WHO, 80% of the people living in rural areas depend on medicinal plants as primary healthcare system. The synthetic anticancer remedies are beyond the reach of common man because of the cost factor. Plant medicines have a vital role to play in the prevention and treatment of cancer, and medicinal plants are commonly available and comparatively economical [52].

Cape gooseberry fruit preparations can be used as a cheaper alternative to the conventional disinfectants. Cape gooseberry fruit is a storehouse of a good variety of compounds (phenolic, flavonoid, and volatile). Latest and previous studies have concluded the beneficial aspects of plant-derived drugs as good source of anticancer activity agents [10]. Flavonoids are known for their immune-modulatory and anti-inflammatory activities, and inhibiting proinflammatory cytokine production and their receptors [53]. Wu *et al*. [54] reported that *P. peruviana* extract has anti-inflammatory activity. Supercritical carbon dioxide extracts of *P. peruviana* contained high levels of flavonoid and phenol. The extract demonstrated strong xanthine oxidase inhibitory effect. It prevented lipopolysaccharide-induced cell cytotoxicity in murine macrophage cells and remarkably blocked the lipopolysaccharide induction of inducible nitric oxide synthase and cyclooxygenase-2 expression [54]. Cellular generation of free radicals has been associated with human disease states, such as inflammatory diseases, neurodegenerative diseases, cancer, and aging [55].

The results of the present study showed that citronellyl acetate possesses the highest concentration of terpenes in Cape gooseberry fruit. Citronellyl acetate, a monoterpene product of the secondary metabolism of plants, has been shown in the literature to possess several biological activities [56]. Ethanol extract of *P. peruviana* inhibits growth and induces apoptotic death of human Hep G2 cells in culture. In addition, it possesses potent antihepatoma activity and its effect on apoptosis is associated with mitochondrial dysfunction [4].

Our results showed that terpenes (monoterpenes and sesquiterpenes) were in the highest concentration compared with other volatile compounds in Cape gooseberry fruit. Terpenes, one of the most extensive and varied structural compounds occurring in nature, display a wide range of biological and pharmacological activities. Terpenes have been shown to provide relevant protection under oxidative stress conditions in different diseases including liver, renal, neurodegenerative, and cardiovascular diseases, cancer, diabetes as well as in aging [57]. Our results showed that isoeugenol was in the highest concentration of all other alcohols in Cape gooseberry fruit [58]. Many studies have explored isoeugenol as an antiproliferative agent against malignant melanoma cells [58]. The literature showed that eugenol possesses antioxidant, antimutagenic, antigenotoxic, anti-inflammatory, and anticancer properties [59–61]. Kim *et al*. [62] and Vidhya and Niramjli [63] demonstrated eugenol-induced apoptosis in human melanoma and breast cancer cells.

In conclusion, Egyptian Cape gooseberry fruits may be suggested as a potential source of natural antioxidant and anticancer agents. This will be important as

an indication of the potentially nutraceutical and economical utility of Cape gooseberry as a new source of bioactive phytochemicals and functional food. Future research should be carried out to evaluate its bioactive effects *in vivo*.

Acknowledgements

The authors extend appreciations to Dr Sameh Rada Hussein, Department of Phytochemistry and Plant Systemic, for his support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Ramadan M, Mörsel JT. Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of golden berry (*Physalis peruviana* L.) juice. J Sci Food Agric 2007; 87:452–460.

2 Strik BC. Berry crops: worldwide area and production systems. In: Zhao Y, editor. *Berry fruit, value-added products for health promotion*. NY, USA: CRC Press; 2007. 5802–5807.

3 Ramadan MF, Mörsel JT. Golden berry: a novel fruit source of fat soluble bioactives. Inform 2004; 15:130–133.

4 McCain R. Golden berry, passion fruit and white sapote: potential fruits for cool subtropical areas. In: Janick J, Simon JE, editors. *New crops*. New York, USA: Wiley and Sons; 1993. 479–486.

5 Rehm S, Espig G. Fruit. In: Sigmund R, Gustav E, editors. *The cultivated plants of the topics and subtropics, cultivation, economic value, utilization*. Weikersheim, Germany: Verlag Josef Margraf; 1991. 169–245.

6 Wu SJ, Ng LT, Chen CH, Lin DL, Wang SS, Lin CC. Antihepatoma activity of *Physalis angulata* and *Physalis peruviana* extracts and their effects on apoptosis in human Hep G2 cells. Life Sci 2004; 74:2061–2073.

7 Wu SJ, Ng LT, Lin DL, Wang SS, Lin CC. *Physalis peruviana* extract induces apoptosis in human Hep G2 cells through CD95/CD95L system and the mitochondrial signaling transduction pathway. Cancer Lett 2004; 215:199–208.

8 Arun M, Asha VV. Preliminary studies on antihepatotoxic effect of *Physalis peruviana* Linn. (Solanaceae) against carbon tetrachloride induced acute liver injury in rats. J Ethnopharmacol 2007; 111:110–114.

9 Mayorga H, Knapp H, Winterhalter P, Duque C. Glycosidically bound flavor compounds of cape gooseberry (*Physalis peruviana* L.). J Agric Food Chem 2001; 49:1904–1908.

10 Ramadan MF. Bioactive phytochemicals, nutritional value, and functional properties of cape gooseberry (*Physalis peruviana*): an overview. Food Res Int 2011; 44:1830–1836.

11 Kataoka H, Lord HL, Pawliszyn J. Applications of solid-phase micro-extraction in food analysis. J Chromatogr 2000; 88:35–62.

12 Parejo I, Viladomat F, Bastida J, Rosas-Romero A, Flerlage N, Burillo J, Codina C. Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants, J Agric Food Chem 2002; 50:6882-6889.

13 Ramadan MM, Abd Algader NN, El-kamali HH, Ghanem KZ, Farrag AH. Chemopreventive effect of *Coriandrum sativum* fruits on hepatic toxicity in male rats. World J Med Sci 2013; 8:322-333.

14 Ramadan MM, Abd-Algader NN, El-Kamali HH, Ghanem KZ, Farrag AH. Volatile compounds and antioxidant activity of the aromatic herb *Anethum graveolens*. J Arab Soc Med Res 2013; 8:79–88.

15 Abd-Algader NN, El-Kamali HH, Ramadan MM, Ghanem KZ, Farrag AH. *Xylopia aethiopica* volatile compounds protect against panadol-induced hepatic and renal toxicity in male rats. World Appl Sci J 2013; 27:10-22.

16 Ramadan MM, Yehia HA, Shaheen MS, Abed El-Fattah MS. Aroma volatiles, antibacterial, antifungal and antioxidant properties of essential oils obtained from some spices widely consumed in Egypt. Am Euras J Agric Environ Sci 2014; 14:486–494.

17 Ramadan MM, Ali MM, Ghanem KZ, El-Ghorab AH. Essential oils from Egyptian aromatic plants as antioxidant and novel anticancer agents in human cancer cell lines. Grasas y Aceites 2015; 66:1–9.

18 El-Ghorab AH, Ramadan MM, Abd El-Moez SI, Soliman AM. Essential oil, anti-oxidant, antimicrobial and anticancer activities of Egyptian *Pluchea dioscoridis* extract. Res J Pharm Bio Chem Sci 2015; 6:1255–1265.

19 Ram MS, Seitz LM, Rengarajan R. Use of an autosampler for dynamic headspace extraction of volatile compounds from grains and effect of added water on the extraction. J Agric Food Chem 1999; 47:4202–4208.

20 Adams RP. *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, IL, USA: Allured Publishing; 1995.

21 Rajeswari G, Murugan M, Mohan VR. GC–MS analysis of bioactive components of *Hugonia mystax* L. (Linaceae). Res J Pharm Biol Chem Sci 2012; 3:301–308.

22 Žilića S, Serpenb A, Akıllıoğluc G, Jankovića M, Gökmen V. Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. J Cereal Sci 2012; 56:652–658.

23 Thaipong K, Boonprakoba U, Crosby K, Cisneros L, Byrnec DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal 2006; 19:669–675.

24 Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem 2001; 73:239–244.

25 Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. Anal Biochem 1996; 239:70–76.

26 Romero D, Gomez-Zapata M, Luna A, Garcia-Fernandez JA. Morphological characterization of BGM (Buffalo Green Monkey) cell line exposed to low doses of cadmium chloride. Toxicol In Vitro 2003; 17:293–299.

27 Mossmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65:55–63.

28 Yilmatekin M. Analysis of volatile components of Cape gooseberry (*Physalis peruviana* L.) grown in Turkey by HS-SPME and GC–MS. ScientificWorldJournal 2014; 1:1–8.

29 Rodríguez-Concepción M, Boronat A. Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. Plant Physiol 2002; 130:1079–1089.

30 Christensen LP, Edelenbos M, Kreutzmann S. Fruits and vegetables of moderate climate. In: RG Berger, editor. *Flavours and fragrances — chemistry, bioprocessing and sustainability*. Berlin, Germany: Springer; 2007. 135–187.

31 Maarse H. *Volatile compounds in foods and beverages*. New York, NY, USA: Marcel Dekker; 1991.

32 Belitz HD, Grosch W, Schieberle P. *Food chemistry*. Berlin, Germany: Springer; 2009.

33 Nunes C, Coimbra MA, Saraiva J, Rocha SM. Study of the volatile components of a candied plum and estimation of their contribution to the aroma. Food Chem 2008; 111:897–905.

34 Schwab W, Schreier P. Enzymatic formation of flavor volatiles from lipids. In: TM Kuo, HW Gardner, editors. *Lipid biotechnology*. New York, NY, USA: Marcel Dekker; 2002. 25–39.

35 Wyllie SG, Fellman JK. Formation of volatile branched chain esters in bananas (*Musa sapientum* L.) J Agric Food Chem 2000; 48:3493–3496.

36 Beekwilder J, Alvarez-Huerta M, Neef E, Verstappen FW, Bouwmeester HJ, Aharoni A. Functional characterization of enzymes forming volatile esters from strawberry and banana. Plant Physiol 2004; 135:1865–1878.

37 Werkhoff P, Güntert M, Krammer G, Sommer H, Kaulen J. Vacuum headspace method in aroma research: flavor chemistry of yellow passion fruits. J Agric Food Chem 1998; 46:1076–1093.

38 Malowicki SM, Martin R, Qian MC. Volatile composition in raspberry cultivars grown in the pacific northwest determined by stir bar sorptive extraction-gas chromatography–mass spectrometry, J Agric Food Chem 2008; 56:4128–4133.

39 Perestrelo R, Fernandes A, Albuquerque FA, Marques JC, Câmara JS. Analytical characterization of the aroma of Tinta Negra Mole red wine: identification of the main odorants compounds. Anal Chim Acta 2006; 563:154–164.

40 Osorio S, Munoz C, Valpuesta V. Physiology and biochemistry of fruit flavors. In: Hui YH, editor. *Handbook of fruit and vegetable flavors*. Hoboken, NJ, USA: John Wiley & Sons; 2010. 24–43.

41 Oke F, Aslim B, Ozturk S, Altundag S. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chem 2009; 112:874–879.

42 Dinan L, Sarker S, Sik V. 28-Hydroxywithanolide E from *Physalis peruviana*. Photochemistry 1997; 44:509–512.

43 Wang IK, Lin SY, Lin JK. Induction of apoptosis by apigenin and related flavonoids through cytochrome *c* release and activation of caspase-9 and caspase-3 in leukemia HL-60 cells. Eur J Cancer 1999; 35:1517–1525.

44 Elliot JG. Application of antioxidant vitamins in foods and beverages. Food Technol 1999; 53:46–48.

45 Matkowski A, Piotrowska M. Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. Fitoterapia 2006; 77:346–353.

46 Li H, Wang XY, Li Y, Li PH, Wang H. Polyphenolic compounds and antioxidant properties of selected China wines. Food Chem 2009; 112:454–460.

47 Narváez-Cuenca CE, Mateus-Gómez A, Restrepo-Sánchez LP. Antioxidant capacity and total phenolic content of air-dried Cape gooseberry (*Physalis peruviana* L.) at different ripeness stages. Agron Colomb 2014; 32:232–237.

48 Bozin B, Mimica-Duki N, Bogavac M, Suvajdzic L, Simin N, Samojlik I, Couladis M. Chemical composition, antioxidant and antibacterial properties of *Achillea collina* essential oils. Molecules 2008; 13:2058–2098.

49 Baraga PC, Dal Sasso M, Fonti E, Culici M. Antioxidant activity of bisabolol: inhibitory effects on chemiluminescence of human neutrophil bursts and cell-free systems. Pharmacology 2009; 83:110–115.

50 Namiki M. Antioxidant/antimutagen in food. Crit Rnl Food Sci Nurr 1990; 29:273–300.

51 Krimat ST, Dob T, Toumi M, Kesouri A, Noasri A. Assessment of phytochemicals, antioxidant, antimicrobial and cytotoxic properties of *Salvia chudaei* Batt. et Trab. endemic medicinal plant from Algeria. J Mater Environ Sci 2015; 6:70–78.

52 Shaban A, Mishra GM, Nautiyal R, Srivastava S, Tripathi K, Chaudhary P, Verma S. In vitro cytotoxicity of *Moringa oleifera* against different human cancer cell lines. Asian J Pharm Clin Res 2012; 5:1–4.

53 Kempuraj D, Madhappan B, Christodoulou S, Boucher W, Cao J, Papadopoulou N, *et al*. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. BrJ Pharmacol 2005; 145:934–944.

54 Wu SJ, Tsai JY, Chang SP, Lin DL, Wang SS, Huang SN. Supercritical carbon dioxide extract exhibits enhanced antioxidant and antiinflammatory activities of *Physalis peruviana*. J Ethnopharmacol 2006; 108:407–413.

55 Yang WL, Addona T, Nair DG, Qi L, Ravikumar TS. Apoptosis induced by cryo-injury in human colorectal cancer cells is associated with mitochondrial dysfunction. Int J Cancer 2003; 103:360–369.

56 Rios ER, Rocha NF, Carvalho AM, Vasconcelos CL. TRP and ASIC channels mediate the antinociceptive effect of citronellyl acetate. Chem Biol Interact 2013; 203:1–9.

57 González-Burgos E, Gómez-Serranillos MP. Terpene compounds in nature: a review of their potential antioxidant activity. Curr Med Chem 2012; 19:5319–5341.

58 Ghosh R, Nadiminty N, Fitzpatrick JE, Alworth WL, Slaga TJ, Kumar AP. Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity. J Biol Chem 2005; 280:5812–5819.

59 Pisano M, Pagnan G, Loi M, Mura ME, Tilocca MG, Palmieri G, *et al*. Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. Mol Cancer 2007; 6:8–20.

60 Ogata M, Hoshi M, Urano S, Endo T. Antioxidant activity of eugenol and related monomeric and dimeric compounds. Chem Pharm Bull 2000; 48:1467–1469.

61 Benencia F, Courreges MC. *In vitro* and *in vivo* activity of eugenol on human herpes virus. Phytother Res 2000; 14:495–500.

62 Kim GC, Choi DS, Lim JS, Jeong HC, Kim IR, Lee MH, Park BS. Caspases-dependent apoptosis in human melanoma cell by eugenol. Korean J Anat 2006; 39:245–253.

63 Vidhya N, Niramjli D. Induction of apoptosis by eugenol in human breast cancer cell. Indian J Biol 2011; 49:871-878.