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Glucosinolates, Glycosidically Bound Volatiles and Antimicrobial Activity of *Brassica oleraceae* Var. Botrytis, (Soultany Cultivar)

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Abstract

Background: Egyptian cauliflower *Brassica oleracea*, L. var. Botrytis L. Soultany cultivar, is an important edible plant in Mediterranean countries. Only a few researches were focused on antimicrobial activity of its volatiles and glucosinolates. Objective: To evaluate the antimicrobial activity of volatiles and glucosinolates of Egyptian Cauliflower and identify them by GC/MS and HPLC/MS designs. **Materials and Methods:** The semi-volatile and volatile constituents of were extracted by hydrodistillation from leaves, stems and inflorescences, using a Likens -Nickerson-type apparatus. The extracts from fresh and frozen vegetables were investigated by GC/MS and HPLC/MS. the volatile samples containing glucosinolate degradation products were evaluated for antimicrobial activity using the disc diffusion method. **Results:** From the fresh leaves extract, a total of 49 compounds were identified, representing 98.79% of the oil. The major constituent was found to be hex-3(Z)-enol (18.86%). From fresh disrupted inflorescence tissues of Egyptian cauliflower 45 compounds were detected, representing 93.37% of the extract. Nonacosane and 11-methoxybenz(a) anthracene-7,12-dione were identified as major constituents of the hydrodistillation products, representing, respectively, 17.7 and 8.31% of the volatiles. From frozen inflorescence tissues, dimethyl trisulfide and butylated hydroxy toluene were detected as predominant components representing 15.88 and 9.69% respectively. In the latter, hex-3(Z)-enol was not found to be the major constituent as happened in fresh leaves, but representing 3.88%. From fresh stem tissues, dimethyl trisulphide was detected as major constituent representing a percentage (24.06%) more than present in frozen inflorescence tissues. Twenty two compounds were identified by triple quad HPLC/MS. Volatile samples expressed a wide range of growth inhibition activity against both Gram-positive and Gram-negative bacteria and fungi, showing the highest inhibitory effects against E .coli and K. pneumonia strains. **Conclusion:** Hydrodistilled compounds present in the stems, leaves and inflorescence tissues of this cultivar has a highly promising antimicrobial activity.

Keywords: Cauliflower, *Brassica oleraceae*, Glucosinolates, GC/MS, HPLC/MS, Antimicrobial activity.

1. Introduction

Glucosinolates (GLSs) are a class of nitrogen and sulfur-containing natural products distributed in 16 dicotyledonous families of the order Capparales (Al-Shehbaz et al, 1987; Verkerk et al; 2009). Mostly glucosinolates-containing genera are clustered within Brassicaceae, Capparaceae and Caricaceae families (Fahey et al., 2001).Upon plant damage by food processing methods, such as physical distruption of plant cells, chewing and high temperature enhance activation of thioglycoside glucohydrolase for hydrolysis and/or thermal degradation and various breakdown products are formed i.e. Isothiocyanates (ITCs), thiocyanates, nitriles, etc. (Figure 1). (Ivica et al., 2010).

GLSs metabolites have been recognized as antimicrobial agents for many decades (Fahey et al., 2001) and this activity has been reported to be a part of a Cruicifers defense against pathogen attack against such and related strains of human pathogens and fungi (Radulovic et al., 2011), and some of these may be present in the soil associated with the plant species and hence could represent direct plant pathogens (e.g. *Aspergillus fumigates* (O'German et al., 2008). Among the degradation products of GLSs, ITCs have been reported to possess broad spectrum biological activity against bacterial and fungal pathogens, nematodes, insects and weeds (Brown et al., 1997; Lin et al., 2000; Manici et al., 2000; Resa et al., 1999). It is known that nitriles are also biologically active, but effects are more limited than are the ITCs (Ivica et al., 2010).

Just recently O-glycosidically-bound volatile compounds have also been reported in plants to the Brassicaceae family (Blozevic et al., 2008 a,b & 2009; Mastelic et al., 2006) rather than have been reported in more than 170 plants belonging to 50 families (Crouzet et al., 1999). Glycosidically-bound volatiles are considered as storage forms of aroma compounds that can cause the antimicrobial or medicinal properties of

plants (Cutler et al., 1996; DeTommasi et al., 1996). These compounds are thermally stable, in contrast to intact GLSs, and are able to release volatile O-aglycons only by acid or enzymatic hydrolysis (Ivica et al., 2010). Released volatiles, such as aliphatic alcohols, phenyl propane derivatives, C13-Norisoprenoids, monoterpenes and sesquiterpenes were known for their biological activities (Ivica et al., 2010).

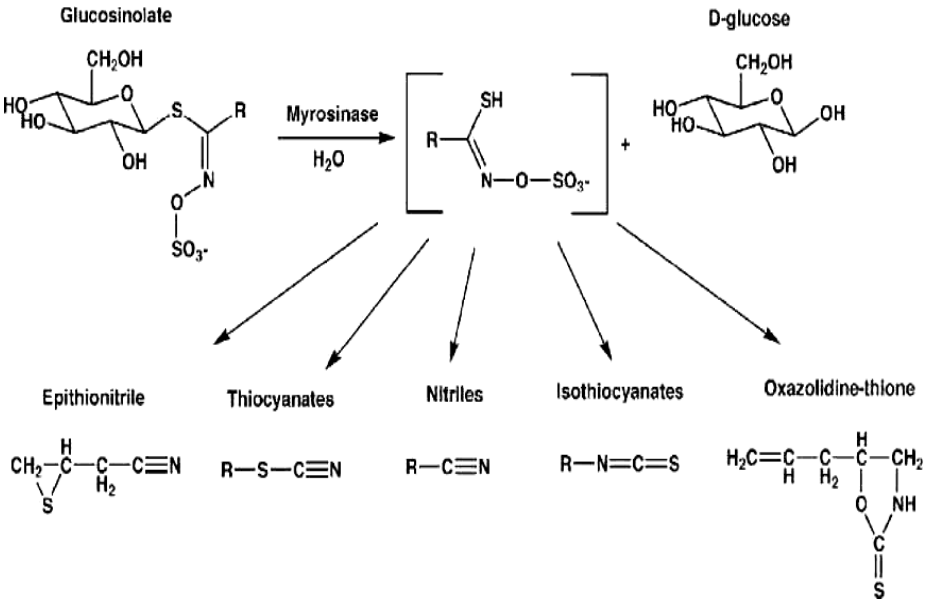


Figure 1: The general structure of glucosinolates and their enzymatic degradation products (adapted from Rask et al. (2000).

Since the end of the sixties, much work has been carried out on the nature of the flavor volatiles of foodstuffs, using a combination of capillary gas chromatography and mass spectrometry as well as investigation of their antimicrobial activities. Cruciferous vegetables are known to contain organic sulfur. Their characteristic flavor and odor have been attributed to volatile sulfur containing compounds. Many studies have been reported, concerning cabbages, cauliflowers, Brussels sprouts and broccoli (Buttery et al., 1976; Daxenbichler et al., 1977; MacLeod et al., 1970c; Marks, et al., 1992; Pearson et al., 1981; Van Langenhove et al., 1991; Wallbank et al., 1976). Composition of volatile constituents has been used to identify new volatile flavoring compounds, such as sulfides or ITCs (Maruyama et al., 1970), to evaluate the effects of variations in cooking methods (MacLeod et al., 1970a) and to compare the volatile fractions from various cruciferous vegetables (MacLeod et al., 1970b). Studies to improve the storage time of cruciferous vegetables (MacLeod et al., 1979) have emphasized the need for an improved knowledge of the compounds contributing to their flavor.

Much work has been carried out on raw material. Some vegetables have been investigated, to analyze preservation effects on volatile compound composition. (MacLeod et al., 1970) reported analytical data on flavor volatile composition of a number of dehydrated cabbage samples, indicating the poor nature of the preserved product as a substitute for the fresh vegetable. Using the same sampling techniques (MacLeod et al., 1970b), the volatile constituents of cooked vegetables were collected and analyzed by gas chromatography, in order to compare them with the fresh material. (MacLeod et al., 1979) have also examined the effect of freezing and blanching on the nature of the volatile flavor components of Brussels sprouts: appreciable variations in the chemical composition of the flavor extracts were observed, and possible reasons for unusual and unacceptably strong flavors of frozen Brussels sprouts were suggested.

In most of these studies, the vegetables were placed in boiling water. Volatile compounds were water-distillated and collected at low temperature (Maruyama et al., 1970; MacLeod et al., 1968, 1970a, 1970b, 1970c, 1979), trapped on an adsorption tube (Van Langenhove et al., 1991) or extracted with solvents (Daxenbichler et al., 1977). Very few studies deal with other techniques, such as hydrodistillation using a Likens-Nickerson-type apparatus (Buttery et al., 1976) or static and dynamic headspace analysis (Wallbank et al., 1976). In each case, sulfides, polysulfides, thiols, isothiocyanates, nitriles, alcohols, carbonyl compounds (ketones, aldehydes, and esters), furans and terpene hydrocarbones were identified in cabbage, cauliflower, Brussels sprouts and broccoli. Dimethyl trisulfide was present as a major aroma component in cooked Brassicaceous vegetables (Maruyama et al., 1970). Moreover, isothiocyanates and nitriles were also found to be predominant in Brussels sprouts, whereas aldehydes were the most abundant cauliflower volatiles (Van Langenhove et al., 1991). For cabbage, evidence based on GC retention times was obtained by (Buttery et al., 1976), 2,3,5-trithiahexane, dimethyl sulfide, dimethyl trisulfide, 2-phenylethyl cyanide and 2- phenylethyl

isothiocyanate were identified as important aroma components.

Much less interest was devoted to Egyptian cauliflower, as this cultivar has been commercialized for less than 15 years. This Egyptian cauliflower variety has a unique flavor and a typical yellowish white, small inflorescence representing only 35- 40% of total weight of fresh plant, while the byproducts (stems and leaves) are considered the major parts. It is not expensive as well as other cultivars grown in Egypt such as Snow ball and Snow crown cultivars and is available anywhere for long time so it is well appreciated by consumers. No spectral evidence has been previously obtained to analyze volatiles isolated from the stems. Actually, no studies concerns volatile extraction from Egyptian cauliflower. Generally, no studies included volatile extraction from byproducts except carried by (Valette et al., 2003): volatile compounds from raw, ripening and frozen florets were extracted by hydrodistillation compared by leaves and analyzed by GC/MS.

The compounds identified in the extract and their importance were described: dimethyl trisulfide, dimethyl disulfide and hex-3(Z)-enol were reported as main volatile constituents in the raw extract of florets, whereas dimethyl trisulfide and dimethyl disulfide only were prevalent in ripening and frozen florets, but, hex 3(Z)-enol represented individually 61.1% of the oil distillated from leaves However, no identification of specific components was carried out in the stems.

The massive increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs, and it necessitated the search for new antimicrobials from alternative sources. Natural compounds have become a source of numerous therapeutic agents. Recent progress to discover drugs from natural sources has resulted in compounds that are being developed to treat resistant bacteria, viruses and immunosuppressive disorders (Amghalia et al., 2009). Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action are also very likely to differ. Much growing interest correlating the phytochemical constituents of a medicinal plant with its pharmacological activity is needed (Prachayasittikul et al., 2008; Nogueira et al., 2008). Screening the active compounds from plants has lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases (Roy et al., 2009). Glucosinolates were previously reported in Cauliflower (Adarsh et al., 2009; Jon et al., 2009; Rozenn et al., 1999) namely, sinigrin, glucoraphanin, , Glucoperin**,** glucobrassicin etc. as seen in table 1. To our knowledge, there is no previous reports on the biological activity of volatiles belonging to Cauliflower (Botrytis variety, *Brassica oleraceae* species). Thus, the aim of this study was to investigate the content of these volatiles using different methods of isolation, namely, hydrodistillation and extraction. Moreover, antimicrobial activity of the isolates containing glucosinolate degradation products was evaluated.

2. Materials and methods

2.1. General experimental procedures:

All chemicals that are commercially available were used as received (Aldrich, USA; Merck, Germany; Fluka, Germany), except that the solvents were purified by distillation. The GC/MS analyses were performed on a Hewlett- Packard 6890N gas chromatograph equipped with fused silica capillary column DB-5 MS (5% phenylmethylsiloxane, 30 m x 0.32 mm, film thickness 0.25 µm, Agilent Technologies, USA) which is coupled with a 5975B mass selective detector from the same company as detailed in 2.4.

2.2. Plant materials:

Egyptian cauliflowers, *Brassica oleracea* L. var. Botrytis L., were obtained from a local farm, Bayaad el Arab, Beni-Swef, Egypt). Botanical identification of the vegetable was authenticated by Prof. Dr. Abdelhalim Mohammed; Flora department, Agricultural museum, Cairo, Egypt. Voucher speciemen were kept in the Agricultural museum. The studied species is commercialized under the name ‘‘Egyptian Soultany’’ cultivar. All cauliflower samples were cut at the same time. One set was analyzed immediately; and one set was frozen at 20^{o}C until analyzed.

2.3. Extraction of volatiles:

This method of preparing the oil extracts was processed as been reported in (Valette et al., 2003) in Romanesco cauliflower but with few changes as following: standard conditions were used for all experiments. Fresh leaves, stems and inflorescences (1kg) were cut into small pieces and placed in a 2 liters flask with 1.5 liter of water. The mixture was subjected to hydrodistillation using a Likens-Nikerson type apparatus. The volatile fraction was extracted under atmospheric pressure during 4 h, using diethyl ether (50 ml) as extracting solvent. This extract was dried over anhydrous sodium sulphate and carefully concentrated to small volume (~ 0.5 ml), with a rotary evaporator at atmospheric pressure. Further, this oil was stored at low temperature prior to analysis. The frozen inflorescences were hydrodistilled, following the method previously described. The frozen vegetables were first thawed using a microwave oven during 10 min (defrozen mode). Before analysis, the extracts were treated as indicated above.

2.4. Capillary GC-mass spectral analysis:

Each concentrated extract sample was analyzed by GC/MS, using Hewlett- Packard-Agilent 6890N system, with a DB-5 (5% phenylmethylsiloxane, 30 m x 0.32 mm fused silica capillary column; film thickness, 0.25 µm) which is coupled with a 5975B mass selective detector. GC oven initial temperature was 40^{o}C and was programmed to 280^{o}C at a rate of 2^{o}C / min., and finally held at 280 ^{o}C during 120 min. Operating conditions of the GC were as follows: helium was used as carrier gas (1 ml/min); the temperature of injector and detector was 250^{o}C and 280^{o}C, respectively; the volume injected was 1µl (diethyl ether solution) in splitless mode. Retention indices were determined compared compared with (Adams et al., 2009; Kovat's index pherobase., 2012; Valette et al., 2003 & 2005; Fatima et al., 2010 & 2009; Al-Gendy et al., 2010; Ivica et al., 2010; Nico et al., 2012) as references. The mass spectra were performed at 70 eV of the mass range of 50-900. No replicates were performed for any sample. The values were determined for each component identified (Table 1).

2.5. HPLC/DAD/MS.MS.MS:

2.5.1. Chemicals:

Methanol, acetonitrile HPLC gradient grade were obtained from Baker (J.T. Baker, Deventer, The Netherlands). Formic acid was purchased from Sigma-Aldrich-Fluka (Madrid, Spain).

2.5.2. Sample treatment:

Cauliflower inflorescences, leaves and stems of *B. oleracea* were packed in PE/aluminum bags under vacuum, frozen at -80^{o}C and analysed within months. Sample extraction was carried out following (Song et al., 2007), with modifications to take into account the flavonoids and the sinapic acid derivatives. Frozen cauliflower samples were minced in a blender mixer Robot-coupe Blixer 3 (Robot Coupe (UK) Ltd., Isleworth UK). An aliquot of 1.5 g of frozen sample was placed in centrifuge tube, followed by 7.5 ml of cold (4^{o}C) methanol to minimize enzymatic activities. Samples were then put in a thermostatic water bath (Memmert GmbH+ Co., KG, Schwabach, Germany) and maintained at 70^{o}C for 15 min. Extracts were refrigerated in an ice water bath and centrifuged at 10,000 rpm for 10 min at 4^{o}C in a Beckman J2-MC centrifuge (Beckman Instruments INC., Palo Alto, CA, USA). An aliquot of 2 mL of the clean supernatant was evaporated to dryness with nitrogen and reconstituted with 1 mL of mobile phase A (10% methanol in 0.2 % formic acid), filtered through a PTFE 0.2 µm filter and injected into the HPLC-MS/MS/MS system under the conditions reported.

2.5.3. HPLC-mass spectral analysis:

The chromatographic system consisted of a Dionex Ultimate 300 HPLC (Bremen, Germany) composed of a quaternary pump with an on line degasser, a thermostated column compartment, a photodiode array detector (DAD), an auto sampler, and Chromelon software. The HPLC separation was performed on Zobrax SB-C18 column (150 mm×4.6 mm, 1.8 µm, Agilent Company, USA), at a flow rate of 0.8 ml/min. The column oven temperature was set at 30^{o}C and the injected volume was 5, 10 and 10 µL of leaves, inflorescences and stems respectively. Mobile phase consisted of two solvents, (A) methanol and (B) 0.2 % formic acid. Separation of compounds was carried out with gradient elution profile: 0 min, A: B 10:90; 36 min, A: B 70:30; 50 min, A: B 100:0; 60 min.

The HPLC-MS system consisted of electrospray ionization (ESI) interfaced Bruker Daltonik Esquire-LC Amazon SL Ion Trap Mass spectrometer (Bremen, Germany) and Dionex Ultimate 300 (Germany), mentioned above. The ionization parameters were as follows: positive ion mode; capillary voltage 4000 V, end plate voltage −500 V; nebulizing gas of nitrogen at 50 p.s.i.; drying gas of 10 l/min nitrogen at 350 °C. Mass analyzer scanned from 15 to 1000 u. The MS/MS/MS spectra were recorded in auto-MS/MS/MS mode. The fragmentation amplitude was set to 1.0 V. MS_3 data were acquired in negative mode.

2.6. In vitro antimicrobial activity:

2.6.1. Test microorganisms:

Nine bacterial strains and four fungal strains used in the present study. The bacteria used were *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024) *Streptococcus pyogenes* (RCMB 010015) as Gram-positive and Gram-negative *Neisseria gonorrhoeae* (RCMB 010076), *Proteous vulgaris* (RCMB 010085), *Klebsiella pneumoniae* (RCMB 0010093), *Shigella flexneri* (RCMB 0100542), *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010056). The fungal strains used were *Aspergillus fumigates* ((RCMB 02564), *Candida albicans* ((RCMB 05035), *Geotricum candidum* (RCMB 05096) and *Trichophyton mentagrophytes* (RCMB 0925). They were obtained from the Regional Center of Mycology and Biotechnology Antimicrobial Unite (RCMB), Cairo, Egypt.

2.6.2. Culture media and antibiotics for antimicrobial assay:

Brain Heart Infusion as liquid and solid media (HiMedia), Mueller- Hinton agar (HiMedia) and Potato Dextrose Agar medium (HiMedia) were used. Ampicilin **(**Oxoid, UK**)** and Gentamycin **(**Oxoid, UK**)** were used

as standard antibacterial agents and Amphotricin B (Sigma Chemical Co., St. Louis, Mo.) was used as a standard antifungal agent.

2.6.3. Determination of the antimicrobial activity:

The antimicrobial activity of oil extractives of inflorescences, leaves and stems of Egyptian cauliflowers, *Brassica oleracea* was screened by the agar disc diffusion method described by Bauer et al., 1966, NCCLS, 1993 and Sahoo et al., 2006 with slight modification. The bacterial cultures were grown in Brain Heart Infusion broth at 37°C. After 6 h of growth, 100 µl of each microorganism at a concentration of $1x10^6$cells/ml was inoculated on the surface of Mueller-Hinton agar plates for bacteria and Potato Dextrose Agar plates for fungi. DMSO with a concentration up to 2% was used to dissolve the oil extracts. Filter paper discs (6 mm in diameter) saturated with 20 µL of the tested essential oils or DMSO (solvent control) were placed on the surface of the inoculated plates. To evaluate the efficiency of the methodology; 50 µl of each essential oil was inserted simultaneously in a hole made in new plates. The plates were incubated at 37 °C for 24 h. The diameter of the inhibition zone was measured in millimeter, and was recorded as mean ± SD of a triplicate experiment. Ampicillin (10 µg), gentamicin (10 µg) discs for bacteria, and amphotericin B (5 µg) for fungi were used as positive standard. Cultured species producing halos equal to or greater than 7 mm were considered susceptible to the tested essential oil. The results are shown in table 3.

3. Results and discussion

3.1. Chemical composition of volatiles:

Volatile compounds and/or constituents that are present in trace amounts may contribute significantly to the characteristic flavor and aroma of fresh, ripening or cooked foods. This investigation was performed to identify the chemical structure of important aroma components in the Egyptian cauliflower "Soultany cultivar''. The volatile fraction was investigated by a GC/MS combination. All the constituents were identified by comparing their mass spectra with those stored in MS databases (Wiley 7N; Mass Finder , Willy7 Nist 05; Mass Finder Libraries), with literature data (Adams, 2009; Valette, 2003), and with home-made mass spectra libraries (built up from pure substances and MS literature data) in addition to retention indices concurrently injected under the same conditions. Identification was confirmed by comparison of their spectra fragmentation pattern with GC data previously published (Davies, 1990; ESO 2000, Valette, 2003).The components of the Egyptian cauliflower extracts, the percentage of each constituent and the retention indices are given in Table 1. Compounds are listed in order of their elution on the DP-5 column.

The chromatographic profile of fresh leaves oil reveals that it contains 51 constituents, representing 98.79%
of the extract. The studied sample was dominated by the unsaturated alcohol fraction that amounted to 30.66% of the oil. In particular, hex-3(Z)-enol (18.86%) was found to be the major constituent of the extract. Other minor compounds were observed, such as dimethyl trisulfide (0.77%), anethol (Z) (3.93%), 3(Z)-hexen-1-ol-acetate (1.31%) and butylated hydroxy toluene (3.99%) , although their proportions were insignificant compared with the alcohols.

The same experiment was conducted with fresh inflorescences. A total of 45 volatile constituents were detected in the sample oil, representing 93.37% of the extract. The volatiles of inflorescences were abundant in alkanes and aromatic ketonic compounds, more exactly in nonacosane (17.7%), 11-methoxybenz[a]-anthracene-7, 12-dione (8.31%) and butylated hydroxy toluene (2.77%). It is noteworthy that 3 sulfur compounds (0.19-0.74%) were also identified, according to the literature, and their mass spectra data. In addition hex-3(Z)-enol was not detected among the volatile constituents of the studied sample compared to the leaves (18.86%).

Comparing the chromatographic profiles obtained with fresh leaves and fresh inflorescence, an interesting fact is noteworthy: the alkenes and aromatic fractions were predominant in Egyptian cauliflower inflorescence, whereas unsaturated alcohols are the major components of the leaves of this vegetable. This has not already been reported by MacLeod et al. (1970a, 1970b): the sulfur volatiles are rather produced by the young part of the cabbage whereas the older leaves liberate a greater proportion of alcohols, aldehydes and ketones or by Valette, 2003: the sulphide fraction is predominant in Romanesco cauliflower inflorescences, whereas unsaturated alcohols were the major components of the leaves of this vegetable. Most of the volatiles liberated from the cauliflower are produced by the inflorescence rather than the outer leaves. Frozen inflorescence was investigated under the same conditions. A total of 45 compounds were detected, representing 99% of the volatiles. This sample was dominated by the sulfur and the phenolic fractions, representing, respectively, 24.47 and 9.69% of the oil. Dimethyl trisulfide has already been reported as the major aroma component in cooked brassicaceous vegetables by Maruyama (1970). The flavour profile of fresh cauliflower is quite different

from that of the preserved vegetable. The decrease of unsaturated alcohols, observed in frozen vegetables, is compensated by a huge increase in polysulfide volatiles. The increased amount of such compounds probably contributes to the characteristic cauliflower flavour, distinguishing the fresh vegetable from the frozen one.

An important variation concerns the ratio of aldehydes produced. It is considerably reduced in fresh cauliflower, whereas it increased in frozen one.

Table 1. Volatile components of *Brassica oleraceae* var. botrytis (Soultany cultivar)

Components	RI[b]	M+ (m/z)	Main fragments (m/z)			Leaves (%)	Inflorescences (%)		Stems (%)
							Fresh (%)	Frozen (%)	
3(E)-Hexen-1-ol	769	100	67.2	56.2	55.2	-	-	0.21	-
Furfural	796	96	69.1	95.1	94.1			tr	
P-xylene	c	106	91.1	67.2	55.2	-	-	tr	0.11
2(E)-Hexanal	823	100	55.1	69.1	95.1	-	-	0.12	-
1-propyl-cycloproane	c	84	56.2	55.2	69.2	-	0.24	-	-
O-xylene	c	106	91.1	106.2	105.2	-	-	-	0.37
3(Z)-Hexen-1-ol	835	100	55.2	67.2	82.2	18.86	-	3.88	-
Allylisothiocyanate	887	99	99.1	91.1	72.1	-	-	-	0.59
Butane-1-isothiocyanate	919	115	115.0	61.1	85.1	-	-	-	tr
Trisulphide dimethyl	935	126	126.0	79.1	111.0	0.77	0.24	15.88	24.06
2,4(E,E)-Heptadienal	947	110	81.1	53.1	110.2	-	-	0.53	tr
2-Pentyl furan	973	138	81.1	57.2	105.1	-	-	0.11	-
3(Z)-Hexene-1-ol-acetate	979	142	67.2	82.2	81.2	1.31	-	-	-
Benzene acetaldehyde	1004	120	91.1	73.2	92.1	-	-	1.01	0.34
7(E)-Methyl-1,6-dioxaspiro[4.5] decane	c	158	84.1	87.1	91.1	-	0.50	-	-
1-(Methylthio)-3-pentanone	1045	132	132.0	57.2	75.1	0.16	-	-	-
Octa-3(E),5(E)-dien-2-one	1057	124	95.1	81.1	79.2	-	-	0.20	0.14
6-(Methylthio)hex-5-ene-3-ol	c	146	73.1	88.1	57.2	0.19	-	-	-
2-methyl-1H-indene	c	130	82.1	54.2	130.1	-	-	-	tr
1-oxo-1.2.4-trithiolane	c	140	61.1	140.1	79.1	-	-	-	1.37
3-Methylthio-hexanal	c	146	70.2	55.2	146.1	0.32	-	-	-
Decamethyl-cyclopentasiloxane*	c	370	355.2	267.1	73.1	-	-	-	0.13
3-Ethylbenzaldehyde	c	134	75.1	134.1	133.1	0.12	-	-	-
2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	c	166	89.1	151.2	107.2	0.10	-	-	-
4-Methyl-3-(2-methylprop-1-enyl)-pyridine	c	147	147.1	116.10	132.10	-	0.18	0.99	-
7-methyl-2.3-dihydroisoindole-1-one	c	147	147.1	116.10	132.10	0.82	-	-	1.96
Dodecamethyl- cyclohexasiloxane*	c	444	341.1	429.20	73.10	-	2.30	-	5.79
Cycloheptane	c	98	55.2	98.20	87.10	-	0.13	-	-
Unknown (1)	e						tr		
Imidazole-4-carboxamide	c	111	111.2	57.2	73.2	-	-	-	tr
(methylthio)-(chloromethylthio) methane	c	142	61.1	93.1	107.1	-	-	-	0.17
Nonanal	1075	142	95.1	57.2	55.2	-	-	0.39	-
1-methyl-N-nitro-2(1H)-pyridinimine	c	153	91.1	75.1	153.1	-	0.64	-	-
Tetradecamethyl –cycloheptasiloxane*	c	519	281.1	73.1	327.0	-	2.43	4.92	6.76
Butylated hydroxyl toluene	c	220	205.3	220.3	206.2	3.99	2.77	9.69	5.63
2,3,5-Trithiahexane	1094	140	61.1	140.1	79.1	-	-	0.67	-
4-(Methylthio)butyl cyanide	1142	129	61.1	115.1	62.1	-	0.19	-	0.25
2-Phenylethyl cyanide	1183	131	91.1	131.1	79.1	-	-	-	tr
Dimethyl tetrasulphide	1187	158	158.0	79.1	64.0	-	-	0.66	2.75
Beta-Cyclocitral	1194	152	137.2	152.2	109.2	0.38	-	-	-
Indole	1248	117	117.1	90.1	89.1	0.11	-	0.30	0.34
Anethol (Z)	1249	148	148.2	147.2	121.1	3.93	0.81	-	0.34
4-Methoxy-Benzaldehyde	1251	136	135.1	136.1	77.1	-	0.22	-	-
Anethol (E)	1282	148	148.2	147.2	117.1	-	0.27	-	0.16
Deca-2(E),4(E)-dienal	1282	152	81.1	117.1	121.1	-	-	0.60	-
Anisyl acetone	1290	178	121.1	164.2	122.1		0.13		
Beta-Damascone	1392	192	177.2	192.2	123.2	tr	-	-	-
Tetradecane	1400	198	57.2	71.2	85.2	-	-	-	tr
2-Phenylethyl isothiocyanate	1419	163	91.1	163.1	105.2	-	-	-	0.19
Trans-b-ionone	1461	192	177.2	123.2	135.2	0.41	-	-	-
8,8-Dimethylspiro(4.6)undecane-6,10-dione	c	208	83.2	55.2	164.2	-	-	-	tr

Table.1: Contin.

5-isopropenyloxymethylene-3,3-dimethyl-cyclohexanone	c	194	163.1	135.1	193.1	-	tr	-	-
Hexadecane	1600	226	57.2	71.2	85.2	-	-	-	tr
1-(isopentylsulfinyl) pentane	c	190	70.2	55.2	146.2	-	-	0.38	-
1.3.5.7-Tetraethyl-1-ethylbutoxysiloxycyclotetrasiloxane*	c	442	355.2	73.1	356.2	-	2.07	-	-
3-methylpyrazolobis(9-borabicyclo[3.1.1]nonane)oxide	c	340	73.2	281.1	221.2	-	-	-	6.08
Guaiol	1600	222	201.2	161.2	149.1	-	-	0.15	-
2,3-dihydro-1,1,3-trimethyl-3-phenyl-1-H-Indene	1716	235	221.2	143.2	222.2	0.41	-	-	-
BHT-quinone-methide	c	218	161.1	203.2	216.2	-	-	tr	0.14
Octadecane	1800	254	57.2	71.2	85.2	-	-	-	tr
2.4-Diphenyl-4-methyl-1(E)-pentene	c	236	119.2	91.1	120.2	0.53	-	-	-
4-(3.4-dimethoxybenzylidine)-1-(4-nitrophenyl)-3-phenyl-2-pyrazolin-5-one	c	429	429.2	73.2	355.1	-	6.0	6.57	5.81
2.4-Diphenyl-4-methyl-2(E)-pentene	c	236	143.2	221.2	236.2	1.25	-	-	-
6.10.14-trimethyl-2-pentadecanone	c	268	58.2	71.2	57.2	-	1.27	0.80	tr
Unknown (2)	f					tr			
4-Cumyl-phenol	c	212	197.2	212.2	55.2	0.26	-	-	-
Cis,cis,cis-7,10,13-hexadecatrienal	c	234	79.1	67.2	91.1	-	-	-	tr
4-t-butyl-2-(dimethylbenzyl)phenol	c	268	175.2	253.2	268.3	0.37	-	-	
Methyl palmitate	1904	270	74.1	87.1	55.2	-	0.43	-	tr
Phytol	1942	296	71.2	57.2	123.2	-	-	0.18	-
Dibutyl isophthalate ester	2025	278	149.1	150.1	223.2	1.71	0.28	0.23	0.28
3,4-dihydro-7,12-dimethylbenz[a]anthracene	c	258	190.2	258.3	95.2	0.45	-	-	-
2-(1,1-dimethylethyl)-4-methyl-1-phenylethyl-phenol	c	268	253.2	149.1	268.3	0.48	-	-	-
Eicosamethyl-cyclodecasiloxane*	c	741	281.1	73.2	147.1	-	2.38	7.09	5.31
Unknown (3)	g					tr			
Dihydro-cis-alpha-copaene-8-ol	c	222	201.2	161.2	81.2	-	0.21		
Hexadecamethyl-heptasiloxane	c	533	355.2	221.1	147.1	-	2.71	1.41	tr
5.7- Diisopropyl xanthone-2-carboxylic acid	c	324	309.3	252.3	324.3	1.53	-	-	-
9.10-Dimethoxy-2.3-dihydroanthracene-1.4-dione	c	270	253.3	268.3	254.2	3.08	-	-	-
Bis[4-diethylamino]phenyl methanone	c	324	309.3	310.3	324.3	5.10	-	-	-
6-aza-5,7,12,14-tetrathiapentacene	c	355	221.1	355.1	281.1	-	5.18	6.88	4.19
Tetracosamethyl-cyclododecasiloxane*		889	355.2	281.1	73.2	-	6.21	3.82	4.65
8b-methyl-2-propyl-2,3,4,5,6,7,8,8b-octahydro-1H-3a-Aza-as-indacene	c	219	204.2	191.2	55.2	-	0.66	-	-
2.3.4.5.6-pentachloro-Benzamine	c	209	55.2	69.2	266.3	1.43	-	-	-
Iron, monocarbonyl-(1.3-butadiene-1.4-dicarbonic acid,diethyl ester)a,a¯ dipyridyl	c	438	355.2	429.2	73.2	-	4.66	5.30	4.56
Heneicosane	2100	296	57.2	71.2	85.2	-	-	0.13	-
Stearic acid	2200	284	55.2	57.2	82.2	-	0.17	-	-
3.3¯dichloro-benzidene	c	252	252.3	185.2	184.2	1.99	-	-	-
Docosane	2200	310	57.2	71.2	85.2	2.03	0.84	-	-
-1,3-dimethyl-pyrido[3,2-d]pyrimidine-2,4-(1H,3H)-dione	c	191	191.2	204.2	106.1	-	0.46		
Benzo(a) phenazine	c	230	230.3	57.2	55.2	-	0.45	-	-
5,14-diol-E,E,Z-1,3,12-nonadecatriene	c	294	55.2	95.2	69.2	0.15	-	-	-
9-(3-butenyl)-anthracene	c	232	119.2	106.2	218.3	-	0.34	-	-
14-beta-pregnane	c	288	95.2	55.2	81.2	0.25	-	-	-
Tricosane	2300	324	57.2	71.2	85.2	3.87	0.81	0.12	-
Bicyclo[3.3.1]nonan-1-ol	c	141	97.1	117.2	115.1			0.28	
Tetracosane	2400	338	57.2	71.2	85.2	5.05	0.59	-	-
Parasiticol	c	302	287.2	302.3	209.2	-	-	-	tr
3-(4-N,N-Dimethylaminophenyl) proponoic acid-2-(diethoxyphosphinyl)-ethyl ester	c	355	221.2	355.2	73.2	-	8.18	4.80	5.45
Dehydroabietic acid	c	300	285.3	239.2	87.2	0.41	-	-	-
Pentacosane	2500	352	57.2	71.2	85.2	5.19	2.37	0.37	-
16-alpha-methylpregnenolone	c	330	315.3	316.3	330.3	7.74	-	-	-

Table.1: Contin.

2-(diphenylmethyl)-1-phenyl-6-hepten-2-ol	c	356	293.3	371.4	372.4	11.46	-	-	-
Hexacosane	2600	366	57.2	71.2	85.2	4.17	1.22	-	-
1-alpha-methyltestoterone	c	314	135.1	191.2	260.3	-	0.55	-	-
6,8-dichloro-2-[4-chlorophenyl]-4-acetylquinoline	c	394	221.2	281.1	147.2	-	-	-	4.36
Heptacosane	2700	380	57.2	71.2	85.2	2.82	-	0.44	-
2,4-di(trimethoxysiloxy)-6,7-(methylene dioxy)-2H-1,4-benzoxazin-3-one-	c	463	341.1	429.2	73.1	-	-	3.08	-
6,6'-biquinoline	c	256	141.2	256.3	55.2	0.26	-	-	-
3-alpha-acetoxy-17-hydroxy androstane	c	334	205.2	274.3	218.3	-	1.40	-	-
Octacosane	2800	394	57.2	71.2	85.2	1.38	1.04	0.10	-
Squalene	c	410	69.2	81.2	95.2	0.29	-	-	-
11-methoxy benz(a)anthracene-7,12-dione	c	288	205.2	288.3	219.3	-	8.31	-	-
Nonacosane	2900	408	57.2	71.2	85.2	0.82	17.07	2.09	0.14
O-terphenyl-13C	c	234	231.2	230.2	119.2	0.31	-	-	-
Triacontane	3000	422	57.2	71.2	85.2	-	0.21	-	-
Annotinine	c	275	117.2	118.2	115.1	0.28	-	-	-
2.4.6-tris(1-methyl-1-phenyl ethyl)-phenol	c	448	433.4	448.4	434.4	0.84	-	-	-
Trapezifolixanthone dimethyl ether	c	406	391.3	406.3	392.3	0.17	-	-	-
3,6,8-tri-tert-butyl-5-methoxyphenanthrene-1,4-dione	c	406	391.3	406.3	392.3		-	0.34	Tr
Muscopyridine	c	231	231.2	117.1	230.2	0.48	-	-	-
N-methyl-Dibenz(E,G) isoindole	c	231	231.2	117.1	230.2	0.52	-	-	-
Octadecamethyl-cyclononasiloxane*	c	667	355.1	73.2	429.2	-	4.66	5.23	0.53
Ethyl 2-[(1-hydroxy-2-isopropyl-5-methyl)cyclohexyl]hexanoate	c	298	355.1	221.1	281.1	-	1.43	-	-
2,610,14-tetramethyl-pentadecane	c	268	71.2	57.2	85.2	-	-	0.25	-
3,4-dihydroxymandelic acid 4TMS	c	472	355.1	73.1	281.1	-	-	5.12	-
Bis(2-ethylhexyl)phthalate	c	390	149.1	167.1	279.2	-	-	0.41	-
Tetradecamethyl-hexasiloxane*	c	458	355.1	221.1	429.2	-	-	2.47	-
4-(Benzylamino)-1,3-diphenyl-5,6,7,8-tetrahydro-2(1H)-quinolinone	c	406	391.3	406.3	392.3	-	-	0.34	-
15-nonacosanone	c	422	225.3	241.3	57.1	-	-	0.11	-
Dodecamethyl-pentasiloxane*	c	384	221.2	355.1	281.1	-	-	0.30	-
Spectra found (%)						**98.79**	**93.37**	**99.13**	**93.72**
Identified components d						**49**	**45**	**46**	**46**
Sulpher containing components (%)						**4 (1.44)**	**3 (5.61)**	**5(24.47)**	**10 (33.64)**
Isothiocyanates (%)						-	-	-	3 (0.83)
Cyanides (%)						-	1 (0.19)	-	2 (0.27)
Alcohols (%)						4(30.66)	2(1.61)	5(4.47)	1 (0.03)
Aldehydes (%)						4(0.92)	1(0.22)	6(2.71)	3 (0.45)
Ketones (%)						8(17.57)	7(16.79	8(11.53)	8 (12.58)
Phenols (%)						5 (5.94)	1 (2.77)	1 (9.69)	1 (5.63)
Esters (%)						2 (3.02)	5(14.98	3(10.33)	3 (4.86)
Ethers (%)						1 (3.93)	2 (1.08)	-	2 (0.50)
Carboxylic acids (%)						2 (1.94)	1 (0.17)	1 (5.12)	-
Miscellaneous (%)						19 (34.66)	22 (49.68)	17 (30.81)	13 (35.2)

[a] Compounds are listed in order of their elution time from a DB-5 column. Compositional values less than 0.1% are denoted as traces (tr)
[b] RI = retention indices as determined on DB-5 column.
[c] Structure confirmed by (Willey7N; Mass Finder and Willey 7 Nist 05; Mass Finder) libraries without determination of their retention indices due to their high molecular weights (> 400);
[d] Identified components less than 0.5% in each organ are not reported.
[e] Unknown (1): 201 (M^+), 111 (100), 57 (82.2), 73 (82), 95 (53.4), 69 (44.4)
[f] Unknown (2): 387 (M^+), 243 (100), 185 (77.3), 55 (77.2), 95 (73.7), 69 (67.3)
[g] Unknown (3): 316 (M^+), 238 (100), 171 (94.8), 170 (68), 119 (53.3), 157 (51.9)
*: Possible artifact due to instrumentation.

The volatile constituents of fresh stems were identified under the same conditions. A total of 45 components were detected, representing 93.9% of the volatiles. This sample was dominated by the sulfur fraction representing 30.62% of the volatiles. As frozen inflorescences, dimethyl trisulphide was considered to be the major constituent, representing 24.06% of the oil. This percentage is more than present in frozen inflorescence (15.88%) whereas the phenolic fraction is reduced in stem tissues. Glucosinolate decomposition leads to the formation of isothiocyanates or cyanides, MacLeod (1970c) suggested that glucosinolates are

decomposed enzymatically, to give isothiocyanates and thermally, to afford the cyanides. The decrease in isothiocyanate ratio is probably due to the inactivation of the enzyme myrosinase during the preservation stage. The increased amount of cyanides is most likely the result of a thermal decomposition of the sinigrin during the processing. The main differences observed between fresh and frozen Egyptian cauliflower are not in good agreement with those already described by MacLeod *et al.*, (1970b) in *B. oleracea* L. var. gemmifera L. and var. Botrytis L and by Vallete, 2003 in Romanesco cauliflower, in that the cyanides and isothiocyanates were identified only in fresh inflorescences and stems of Egyptian cauliflower. Allyl isothiocyanate, 4-(methylthio) butyl cyanide, 2-phenylethyl cyanide, butane-1- isothiocyanate and 2-phenylethyl isothiocyanate were identified in fresh stems, whereas 4-(methylthio) butyl cyanide alone was identified in fresh inflorescences. These components were not identified absolutely in leaves and inflorescences of Egyptian cauliflower with unknown reasons.

3.2. HPLC–mass spectral analysis

On the basis of their triple quad fragmentation ions and UV−Vis spectra, HPLC-DAD-MS/MS/Ms method was used for determination of the major glucosinolates, phenol compounds and phytoalexines in *B. oleraceae* samples and extraction procedure by an HPLC separation with trible detection (DAD and MS/MS/MS) were optimized to allow a suitable evaluation of these nutritionally relevant compounds in cauliflower samples.

Table 2: HPLC/MS identified components of *Brassica. oloreacea* var. botrytis (Soultany cultivar).

List of compounds evaluated by ESI	Rt (min)	ESI transition (m/z) $MS_1/MS_2/(MS_3)$	Time window (min)	Dwell time (s)	Cone voltage (V)	Collision energy (eV)	Relative % (L/F/S)
Glucoraphanin (**6**)	2	436/ 372/ (341/ 258)	0.53–5.00	0.1	34	32	**-/-/tr**
Glucoiberin (**5**)	2.1	423/261/215/114	0.51–4.00	0.1	44	24	**-/0.4/2.2**
Sinigrin (**1**)	2.3	358/259/241/209	0.52–4.00	0.1	38	22	**tr/-/1.2**
Glucoiberverin (**11**)	2.7	406/ 370/ (334/ 272)	2.10–7.00	0.1	40	25	**-/tr/3.1**
Gluconapin (**2**)	3.3	372/ 336/ (226/188)	0.54–8.00	0.1	40	20	**-/tr/3.2**
Kaempferol-3-diglucoside-7-glucoside (**13**)	3.5	771/609/ (429/284)	2.00–16.00	0.1	35	30	**2.9/-/-**
Kaempferol-3-triglucoside-7-glucoside (**14**)	4.4	933/771/ (609/429/284)	2.00–16.00	0.1	35	30	**2.7/-/-**
Glucoerucin (**4**)	4.5	420/ 285/ (198)	2.10–8.00	0.1	48	23	**-/-/1.2**
Kaempferol -7-*o*-glucoside (**15**)	4.7	447/401/ (327/309)	2.13–8.00	0.1	48	30	**7.4/0.1/2.2**
Kaempferol -3-*o*-(sinapoyl)-sophoroside-7-o-glucoside (**16**)	5	977/815/609/429/284	7.00–11.00	0.1	35	30	**1.2/-/-**
Glucobrassicin (**8**)	5.1	447/259/ (238/169/139)	7.40–11.00	0.1	35	25	**-/tr/1.3**
4-methoxy-glucobrassicin (**10**)	5.8	477/259/168/139	10.00–13.00	0.1	35	25	**2.2/-/3.2**
Rutin (**17**)	5.9	609/429/254/151	9.00–16.00	0.1	44	40	**2.1/-/-**
Neoglucobrassicin (**9**)	7.3	477/ 446/417/383	13.00–17.00	0.1	35	25	**6.7/-/7.3**
1-sinapoyl-2-feruloyldiglucoside (**19**)	9.4	723/499/259/193	13.20–17.00	0.1	35	27	**1.3/-/-**
Caulilexin C (**22**)	9.6	186/168/ (-)	3.30–8.00	0.1	37	20	**-/0.5/0.1**
Glucotropaeolin (**7**)	16.6	408/ 372/ (341/ 311)	3.60–8.00	0.1	42	23	**-/tr/-**
n-Hexyl/methylpentyl glucosinolates (**12**)	29.5	402/ 194/179	7.00–11.00	0.1	35	25	**2/0.9/3.8**
Chlorogenic acid (**21**)	29.6	354/176/163	7.20–11.00	0.1	45	26	**3.1/0.9/3.1**
Apigenin (**18**)	32.9	270/225/ (196)	0.55–4.00	0.1	35	30	**-/1.4/-**
Progoitrin (**3**)	35.8	388/280/ (253/237)	0.55–4.00	0.1	35	25	**-/tr/tr**
1,2,2'-Trisinapoyldiglucoside (**20**)	48.6	960/ 913/ (635/ 379)	0.56–4.00	0.1	41	29	**-/tr/-**

Twelve glucosinolates were identified as Sinigrin (2-Propenyl glucosinolate) [**1**], Gluconapin (But-3-enyl glucosinolate) [**2**], Progoitrin (2-Hydroxybut-3-enyl glucosinolate) [**3**], Glucoerucin (4-Methylthiobutyl glucosinolate) [**4**], Glucoiberin (3-Methylsulfinylpropyl glucosinolate) [**5**], Glucoraphanin (4-Methylsulfinylbutyl glucosinolate) [**6**], Glucotropaeolin (Benzyl glucosinolate) [**7**], Glucobrassicin (Indol-3-ylmethyl glucosinolate) [**8**], Neo-glucobrassicin (1-Methoxyindol-3-ylmethyl glucosinolate) [**9**], 4-Methoxyglucobrassicin (4-Methoxyindol-3-ylmethyl glucosinolate) [**10**], Glucoiberverin (2-thiapentayl glucosinolate) [**11**] and n-hexyl glucosinolate (methylpentyl glucosinolate) [**12**] (Figure 3A). In addition, six

flavonoids were also identified, namely Kaempferol-3-diglucoside-7-glucoside [**13**], Kaempferol-3-triglucoside-7-glucoside [**14**], Kaempferol -7-*o*-glucoside [**15**], Kaempferol -3-*o*-(sinapoyl)-sophoroside-7-*o*-glucoside [**16**], Rutin [**17**] and Apigenin [**18**] (Figure 3B).. In a similar way, three Phenolic acids and/or glycosides were identified, namely 1-sinapoyl-2-feruloyldiglucoside [**19**], 1,2,2'-trisinapoyldiglucoside [**20**] and chlorogenic acid [**21**] (Figure 3C) beside one phytoalexine namely Caulilexin C (1-methoxyindol-3-ylacetonitrile) [**22**] (Figure 3 D).

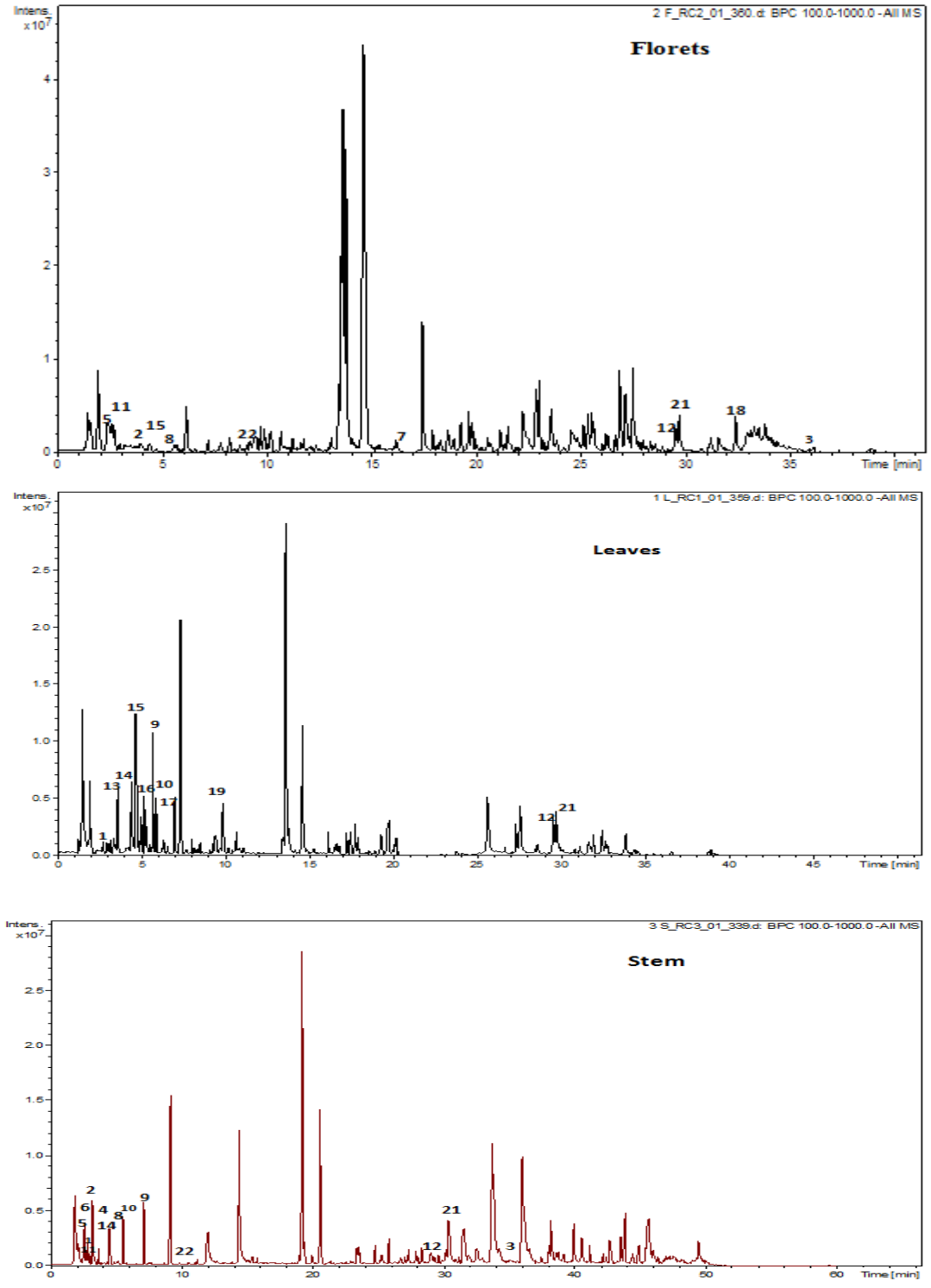
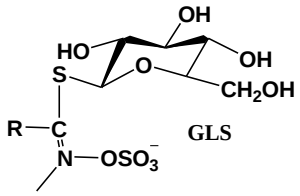


Figure 2: HPLC/MS charts of *Brassica oleraceae* var. botrytis (Soultany cultivar)

A. Glucosinolates:

Basic structure: **GLS** (β-thioglucoside-N- hydroxyl sulfate moiety)



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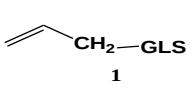

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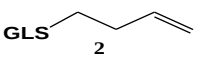

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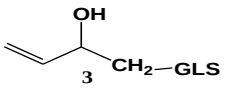

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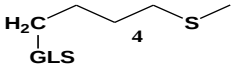

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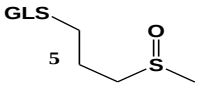

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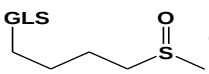

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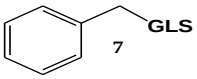

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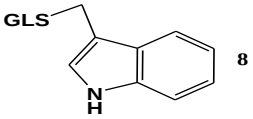

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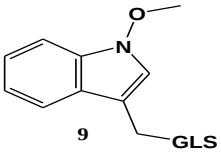

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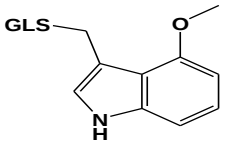

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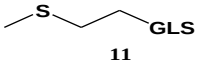

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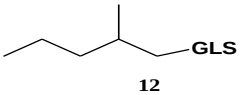
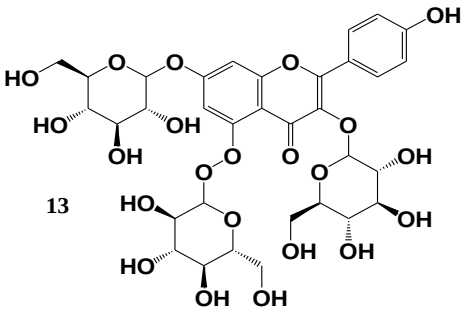


12

B. Free flavonoid and/or glycosoids:



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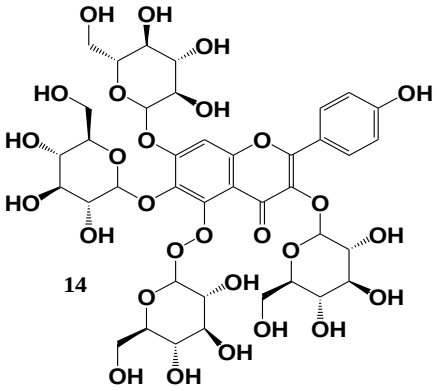

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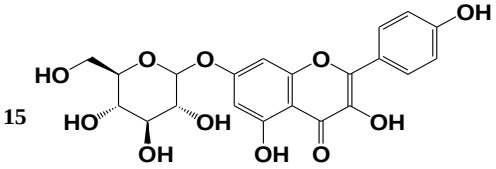

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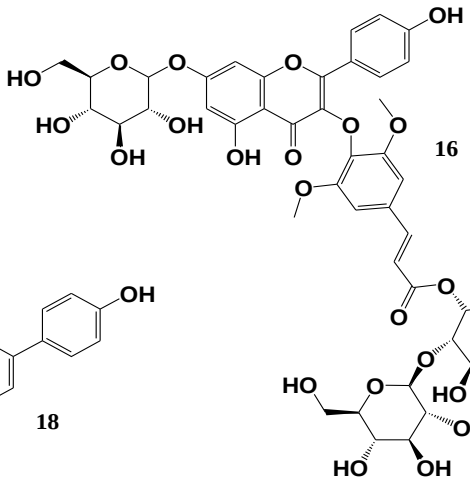

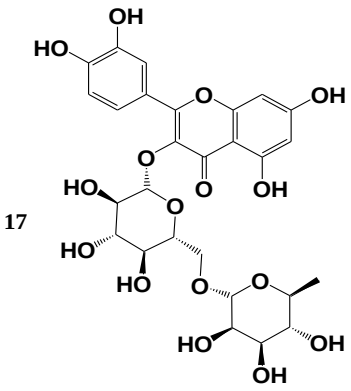

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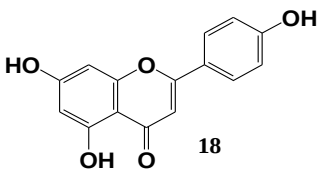
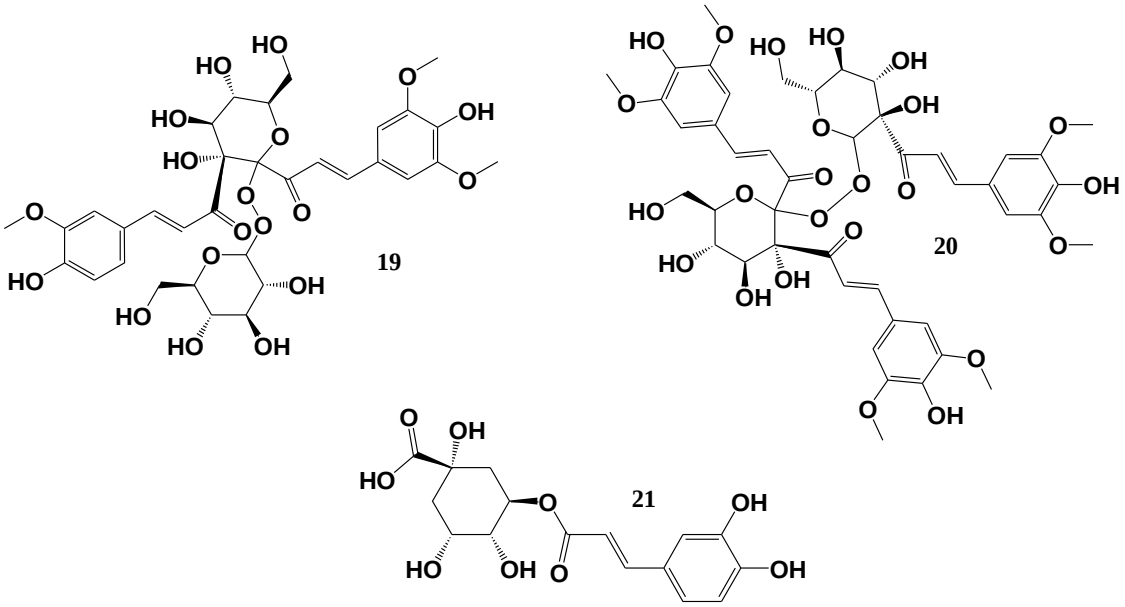


18

C. Free Phenolic acids and/or glycosides:



D. Phytoalexins:

Caulilexin C

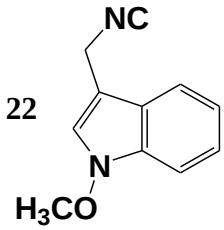


Figure 3: HPLC/MS identified constituents of *Brassica oleraceae* var. botrytis (Soultany cultivar)
(**A**: Glucosinolates; **B**: Free flavonoid and/or glycosoids; **C**: Free Phenolic acids and/or glycosides;**D**: phytoalexin)

The concentrations of glucosinolates, phenolic compounds and phytoalexines found were determined and found to be variable slightly with previously published data of Song *et al*., 2005 and Tian *et al*., 2005. This slight variation can be attributed to the environmental conditions and the consequence of the different origin of our samples.

The glucosinolate profile of *B. oleracea* stems was marked by high relative levels of glucoiberin (2.2%), sinigrin (1.2%), glucoiberverin (3.1%), gluconapin (3.2%), glucoerucin (1.2%), glucobrassicin (1.3%), 4-methoxy-glucobrassicin (3.2%), neoglucobrassicin (7.3%) and n-hexyl glucosinolate (3.8%) whilst the leaves were richer in neoglucobrassicin (6.7%) and n-hexyl glucosinolate (2.0%) and mainly rich in flavonoids Kaempferol-3-diglucoside-7-glucoside (2.9%), Kaempferol-3-triglucoside-7-glucoside (2.7%), Kaempferol -7-*o*-glucoside (7.4%), Kaempferol -3-*o*-(sinapoyl)-sophoroside-7-*o*-glucoside (1.2%) and Rutin (2.2%) meanwhile, inflorescence were poor in glucosinolates and contained (1.4%) apigenin flavonoid and this in agreement with previously reported studies (Branca *et al*., 2002; Volden *et al*., 2009). The results are shown in table 2 and figures 2, 3.

3.3. Antimicrobial activity

The antimicrobial activity of *B. oleraceae var.botrytis*, Egyptian group, Soultany cultivar volatile samples, obtained by hydrodistillation followed by extraction evaluated by measuring the diameters of the inhibition zones for all samples at the volume of 20 µl per disc. The results presented in table 3 show that the volatile samples isolated by hydrodistillation followed by extraction exhibited antimicrobial activity, but not against all microorganisms tested. These volatile samples show the highest inhibitory effects against *E. coli* and *K. pneumonia*, which are well known as naturally resistant to many antimicrobial agents. The volatile sample of stems showed the strongest antimicrobial activity against *E. coli* with a mean zone of inhibition diameter of 19.7±1.37 mm. All other tested Gram-negative bacteria also displayed variable degrees of susceptibility

against all volatile samples, with a range of 11.9±0.83- 16.7±1.15 mm zone of inhibition diameter with some exceptions: *P. aeruginosa* did not display any degree of susceptibility against all volatile samples; neither inflorescences nor leaves volatile samples exhibited antimicrobial activity against *P. vulgaris*; leaves volatile oil individually had no antimicrobial activity against *N. gonorrhoeae*. All tested Gram-positive bacteria were also sensitive against volatile samples isolated by hydrodistillation followed by extraction except for leaves sample with zone of inhibition diameter ranging from 13.8±1.33 to 19.2±1.29 mm. Gram-positive *S. epidermidis* was the most sensitive to volatile sample of stems containing the highest percentage of sulfur compounds obtained by hydroditillation of fresh plant material, with a mean zone of inhibition of 19.2±1.29 mm (Bauer *et al*., 1966) was in a good agreement with these results upon using benzoyl peroxide-sulfur cream for acne vulgaris against *S. epidermidis* strain. These volatile samples were also found to inhibit the growth of fungal organisms (*C. albicans*, *G. candidum*, *T. mentagrophytes* and *A. fumigatus*) with zone of inhibition diameter values ranging from 9.3±0.52 to 19.7±1.29 mm.

Table 3: Antimicrobial activity of volatiles of inflorescences, leaves and stems of *Brassica oleraceae*.

Tested microorganisms / Sample	Inflorescences (fresh+ frozen)	Leaves	Stems	Standard
Fungi				**Amphotericin B**
Aspergillus fumigatus (RCMB 02564)	18.4 ± 1.39	NA	19.7 ± 1.29	24.6± 0.10
Candida albicans (RCMB 05035)	12.1 ± 1.42	9.3 ± 0.52	14.4 ± 1.68	21.8± 0.12
Geotricum candidum (RCMB 05096)	13.3 ± 0.95	10.1 ± 0.75	12.4 ± 1.31	26.4± 0.20
Trichophyton mentagrophytes (RCMB 0925)	15.1± 1.21	12.8± 0.91	18.2 ± 1.19	25.4± 0.16
Gram-positive bacteria:				**Ampicillin**
Staphylococcus aureus (RCMB 010027)	13.8 ± 1.33	NA	16.6 ± 0.71	28.6± 0.14
Staphylococcus epidermidis (RCMB 010024)	15.3 ± 0.92	NA	19.2 ± 1.29	25.2± 0.18
Streptococcus pyogenes (RCMB 010015)	16.3 ± 1.39	NA	17.1 ± 1.12	26.4± 0.34
Gram-negative bacteria:				**Gentamycin**
Neisseria gonorrhoeae (RCMB 010076)	15.9± 0.79	NA	15.5 ± 1.01	19.4± 0.18
Proteous vulgaris (RCMB 010085)	NA	NA	11.9± 0.83	23.4± 0.3
Klebsiella pneumoniae (RCMB 0010093)	17.6 ± 0.89	18.5 ± 1.32	17.4 ± 1.09	26.2± 0.15
Shigella flexneri (RCMB 0100542)	16.3 ± 1.17	16.7 ± 1.15	15.4 ± 1.28	24.8± 0.24
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	NA	17.3± 0.12
Escherichia coli (RCMB 010056)	18.5 ± 0.92	17.2 ± 1.17	19.7 ± 1.37	25.3± 0.18

The test was done using the agar disc diffusion technique, disc diameter: 6.0 mm, RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms. NA: No activity, data are expressed in the form of mean ± SD.

Since *B. oleraceae* species, *Botrytis variety* volatile isolates contain high percentages of glucosinolates and glucosinolate degradation products, as also do many plants belonging to family Brassicaceae, this investigation confirms the importance of studying the correlation between chemical composition of volatiles and antimicrobial activity.

4. Conclusion:

This is the first report concerning the hydrodistilled compounds present in the stems, leaves and inflorescence tissues of this cultivar.

To our knowledge, there are no previous reports on the biological activity of volatiles belonging to Cauliflower (*B. oleraceae* species, *Botrytis variety*). The previous reports showed antimicrobial activities of volatile isolates obtained from other varieties of *Brassica oleraceae* species such as reported by (Sousa *et al*., 2008), or species of different genus that belong to the same family, such as *Eruca sativa* (Khoobchandani *et al*., 2010), *Wasabia japonica* (Shin *et al*., 2004), *Pseudocytisus integrifolius* (Bendimerad *et al*., 2005), *Raphanus sativus* (Beevi *et al*., 2009; Rani *et al*., 2008) and *Erysimum chorintum* (Al-Gendy *et al*., 2010).

Regardless of the isolation method (hydrodistillation and extraction) and differences in volatiles composition, all isolates exhibited different degrees of antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. Also, there were no significant differences in antimicrobial activity among all volatile samples against most microorganisms tested. An exception was noticed with leaves volatiles which were inactive against most microorganisms tested. Glucosinolate degradation products, isothiocyanates and nitriles

may be responsible for the observed antimicrobial potency, as they constitute most of the volatile samples of *B. oleraceae*.

The use of *B. oleraceae* stems and leaves as waste by-products may be considered not only as nutrient but also could be utilized as sources of beneficial antimicrobial agent rather than disposable products.

Cauliflower by-products could be used in ready to eat snacks by using stem and leaves up to 10% after being washed and ground and added to wheat flour.

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