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Volatile composition, antidiabetic, and anti-obesity potential of *Brassica incana* leaf and flowering top extracts

Maria Fernanda Taviano^a (), Sonia Núñez^{b,c}, Adrián Millán-Laleona^{b,c}, Concetta Condurso^d (), Antonella Verzera^d (), Maria Merlino^d (), Monica Ragusa^e, Natalizia Miceli^a () and Víctor López^{b,c}

^aDepartment of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy; ^bDepartment of Pharmacy, Faculty of Health Sciences, Universidad San Jorge, Villanueva de Gállego (Zaragoza), Spain; ^cInstituto Agroalimentario de Aragón-IA2, CITA-Universidad de Zaragoza, Zaragoza, Spain; ^dDepartment of Veterinary Sciences, Viale Palatucci, University of Messina, Messina, Italy; ^eIRCCS Istituto Ortopedico Rizzoli, Complex Structure of Surgical Sciences and Technologies, Bologna, Italy

ABSTRACT

Context: *Brassica incana* Ten. (Brassicaceae) is an edible plant with very limited available information. Previous studies have demonstrated the polyphenolic profile and the antioxidant and cytotoxic properties of the leaf and flowering top hydroalcoholic extracts.

Objective: The volatile composition and the antidiabetic and anti-obesity potential of *B. incana* leaf and flowering top extracts have been investigated.

Material and methods: The volatile characterization of the extracts was attained by HS-SPME-GC/MS analysis. The antidiabetic and anti-obesity potential was investigated spectrophotometrically *in vitro* by the ability to modulate pancreatic lipase and α -glucosidase at different concentrations using orlistat and acarbose as reference drugs. The inhibition of advanced glycation end-products (AGEs) was measured with aminoguanidine as reference and the antioxidant activity with the xanthine/xanthine oxidase system and Trolox for comparative purposes.

Results: Several volatiles belonging to different chemical classes were identified, being sulphur compounds the most abundant in both leaf and flowering top extracts (56.33% and 64.40% of all volatiles). Although the leaf extract showed lower IC₅₀ values in most of the assays (0.968 and 1.921 mg/mL for α -glucosidase; 0.192 and 0.262 mg/mL for AGEs; 0.022 and 0.038 mg/mL for superoxide scavenging), there were no statistically significant differences between both samples. These extracts showed a similar behaviour to Trolox in the xanthine oxidase assay (IC₅₀ values of 0.022 mg/mL for leaf extract; 0.038 mg/mL for flowering top and 0.028 for Trolox).

Conclusions: Leaves and flowering tops from *B. incana* can be used as sources of functional compounds that could act as antidiabetic and anti-obesogenic agents.

ARTICLE HISTORY

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KEYWORDS

Anti-glucosidase; anti-lipase; antioxidant; Brassicaceae; enzyme inhibitor; isothiocyanates; volatile compounds

Introduction

Brassica incana Ten., a wild *B. oleracea*-related species, is an edible plant belonging to the Brassicaceae family. *Brassica incana* is a suffrutex up 100 cm high, woody at the base, branched, glabrous except at base. Basal leaves with petiole with two irregularly dentate wings, are pubescent to tomentose especially on the lower surface and along the veining, ovate to lanceolate, lyrate; the lamina has margin entire and irregularly crenate or 1–2 lobes in the lower half, usually obtuse; upper leaves are denticulate, with amplexicaule basal auricles, gradually smaller. The flowers are gathered in racemes many-flowered with yellow spatulate petals. The fruit is a siliqua patent, constricted at intervals, terete, gradually attenuate into beak (Heywood 1964). This species is native to south-eastern Europe, including Albania, Bosnia-Herzegovina, Croatia, Greece, and Italy; the plant has also been introduced in Ukraine and Crimea (Marhold 2011).

As far as we know, only a few studies on *B. incana* are present in the published literature, which focus on the glucosinolates contained in the leaves and the seeds, while no studies on its therapeutic potential are available (Horn and Vaughan 1983; Heaney et al. 1987; Velasco and Becker 2000).

Considering the very limited information about *B. incana*, our research team started a study aimed at investigating the potential of this species as a source of bioactive phytochemicals. In a previous study, some of the authors of the present work had characterized the volatile composition of *B. incana* fresh leaves and roots (Tripodi et al. 2012). In a recent work, the anti-oxidant properties, the cytotoxicity against human colorectal adenocarcinoma (Caco-2) cells and the absence of toxicity versus brine shrimp larvae (*Artemia salina* Leach) of the hydroalcoholic extracts obtained from the leaves and the flowering tops of *B. incana* grown wild in Sicily (Italy) were established (Miceli et al. 2020). Moreover, the quali-quantitative characterization of the

CONTACT Natalizia Miceli 🖾 nmiceli@unime.it 💽 University of Messina, Messina, Italy; Víctor López 🖾 ilopez@usj.es 🗈 Department of Pharmacy, Faculty of Health Sciences, Universidad San Jorge, Villanueva de Gállego (Zaragoza), Spain

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phenolic compounds was performed, highlighting the presence of quercetin, kaempferol and isorhamnetin derivatives, whose antioxidant, anti-inflammatory properties as well as protective against metabolic disorders have been previously demonstrated (Carullo et al. 2017; Nasri et al. 2017; Liu et al. 2021).

As a continuation of the ongoing research, this work was designed to further investigate the phytochemical volatile profile and the biological potential of the same extracts obtained from edible parts of this species. To achieve a comprehensive view of the volatile composition of the extracts the headspace solid-phase microextraction (HS-SPME) coupled to gas chromatographymass spectrometry (GC-MS) was utilized. Besides, *B. incana* extracts were investigated *in vitro* as candidates with antidiabetic and anti-obesity potential. For this purpose, the ability to modulate lipase and α -glucosidase together with inhibition of advanced glycation end-products (AGEs) and free radicals was examined (Sriramavaratharajan and Murugan 2018; Mustafa et al. 2022).

Materials and methods

Reagents and chemicals

All enzymes for bioassays such as lipase, α -glucosidase and xanthine oxidase were obtained from Sigma-Aldrich (Madrid, Spain). Orlistat as drug reference was also acquired from Sigma-Aldrich (Madrid, Spain) while acarbose was bought in Cymit Quimica (Barcelona, Spain). All other reagents, unless indicated, were purchased from Sigma (St. Louis, MO).

Plant material and extraction procedure

The plant material was collected around Capo d'Orlando (Messina, Italy). The leaves of *Brassica incana* were harvested in November 2018 and the flowering tops in May 2019. The taxonomic identification was confirmed by Prof. S. Ragusa, Department of Health Sciences, University Magna Graecia of Catanzaro. A voucher specimen (1108/18) was deposited in the same department.

After harvesting, the plant material was washed, blended, frozen, and lyophilized. The extraction was carried out as reported in our previous work (Miceli et al. 2020). The yields of the leaf and flowering top hydroalcoholic (70% MeOH) extracts, compared to 100 g of lyophilized plant material, were 26.47% and 33.16%, respectively.

Characterization of volatile compounds by SPME-GC/MS

Extraction (HS-SPME)

The hydroalcoholic extracts of both the leaves and the flowering tops of *B. incana* were analyzed for their volatile composition by HS-SPME-GC/MS.

The dried extracts were solubilized in saturated sodium chloride solution to a final concentration of 10 mg/mL; then 3 ± 0.1 mL of each extract solution were transferred to a 7 mL vial closed with a 'mininert' valve (Supelco, Bellefonte, PA). For the volatile extraction, the sample was equilibrated for 15 min at 40 °C, and a DVB/CAR/PDMS fibre, 50/30 µm film thickness (Supelco, Bellefonte, PA), was exposed for 15 min to the headspace of the sample maintained at 40 °C under continuous magnetic stirring. Finally, the SPME fibre was placed for 3 min into the injector port of the GC/MS, held at 260 °C, for the thermal desorption of the analytes onto the capillary GC column.

Analysis (GC/MS)

The volatiles were analyzed by a Shimadzu GC 2010 Plus gas chromatograph coupled to a TQMS 8040 triple quadrupole mass spectrometer (Shimadzu, Milan, Italy). Two capillary columns of different polarity were used: (1) VF-WAXms, 60 m, 0.25 mm i.d., 0.25 μ m film thickness polar column (Agilent Technologies Italia S.p.A., Milan, Italy); (2) DB-5ms, 30 m, 0.25 mm i.d., 0.25 μ m film thickness apolar column (Agilent Technologies Italia S.p.A., Milan, Italy).

The conditions were as follows. Injection mode: splitless. Oven temperature: (1) 45 °C held for 5 min, then increased to 80 °C at a rate of 10 °C/min and to 240 °C at 2 °C/min, held at 240 °C for 5 min, for VF-WAXms column; (2) 45 °C increased to 160 °C at a rate of 3 °C/min and to 260 °C at 10 °C/min, held at 260 °C for 5 min, for DB-5ms column. Carrier gas: helium at a constant flow of 1 mL/min. Transfer line temperature: 250 °C. Acquisition range: 40–360 *m*/*z*; scan speed of 1250. For the identification of the volatiles, mass spectral data, NIST' 14 (NIST/ EPA/NIH Mass Spectra Library, version 2.0, USA) and FFNSC 3.0 database, linear retention indices (LRI), literature data and injection of the available standards were used (Cincotta et al. 2018).

Bioactivity of B. incana extracts

All *in vitro* bioactivity tests were performed as previously described in Taviano et al. (2020) using control wells with all reagents except for extract and sample wells in order to check the inhibitory profile. Blank wells were also measured in order to eliminate interferences. A wide range of concentrations (0.0001–10 mg/mL for enzymatic assays and 0.03–0.5 mg/mL for AGES) was tested in the assays.

Pancreatic lipase inhibition

Lipase inhibition was measured as previously reported by Taviano et al. (2020) in 96-well microplates. Briefly, $40 \,\mu\text{L}$ of enzyme (2.5 mg/mL in 0.1 M phosphate buffer, pH 7.0), previously centrifugated at $2000 \times g$ for 7 min was mixed with $40 \,\mu\text{L}$ of extract and $20 \,\mu\text{L}$ of 10 mM *p*-nitrophenyl butyrate (*p*-NPB). After 10 min incubation, absorbance was recorded at 405 nm using also orlistat as drug reference.

α -Glucosidase inhibition

 α -Glucosidase inhibition was also investigated as reported by Taviano et al. (2020). 100 µL of enzyme (1 U/mL) dissolved in buffer (12.5 mM Na₂HPO₄, 3.3 mM NaH₂PO₄; pH = 6.9) was mixed with 50 µL of extract and then incubated at room temperature for 10 min. Then, 50 µL of 3 mM *p*-nitrophenyl- α -D-glucopyranoside (pNPG) were added. After 15 min at 37 °C, absorbance was recorded at 405 nm using acarbose as drug reference.

Advanced glycation end-products inhibition

Advanced glycation end-products (AGEs) inhibition was measured in 96-black well-plates according to Spínola and Castilho (2017). 10 mg/mL Bovine serum albumin solution (50 μ L) was mixed with 80 μ L of 0.1 M phosphate buffer (containing sodium azide 3 mM, pH =7.4), 50 μ L of 0.5 M fructose solution (0.5 M) and 20 μ L of extracts. After 24 h incubation at 37 °C in the dark, fluorescence was measured (355 nm excitation wavelength and

460 nm emission wavelength) using aminoguanidine (AMG) as drug reference.

Antiradical activity

Free radical scavenging activity was evaluated by calculating the percentage of inhibition of superoxide radicals generated by xanthine oxidase (Mustafa et al. 2022) using trolox as reference substance. Briefly, 240 μ L of the reaction mixture [90 μ M xanthine, 16 mM Na₂CO₃, and 22.8 μ M nitroblue tetrazolium chloride (NBT) in phosphate buffer pH 7.0] was mixed with 30 μ L of extract solution at different concentrations; then, xanthine oxidase (XO) was added, and absorbance was read at 560 nm after 2 min incubation at 37 °C.

Data and statistical analyses

Results about bioactivity are presented as mean values and standard error of mean (SEM) of at least three independent experiments in different days. All bioactivity assays were performed at between 5 and 9 different concentrations for non-linear regression. GraphPad Prism v.7.0 (GraphPad Software, La Jolla, CA) was used for formal analyses. IC_{50} values were obtained by non-linear regression and one-way ANOVA with Tukey multiple comparison test was used in order to detect differences between the samples.

Results and discussion

Characterization of volatile compounds by SPME-GC/MS

The volatile composition of the hydroalcoholic extracts of the *B. incana* leaves and flowering tops are reported in Tables 1 and 2, respectively. A large number of compounds belonging to the chemical classes of esters, alcohols, acids, ketones, aldehydes, terpenes, hydrocarbons, sulphur compounds and nitriles were detected in the headspace of leaf and flowering top extracts.

Regarding the leaf extract, its volatile fraction was constituted mainly of sulphur compounds (sulphides and isothiocyanates) which accounted for over 56% of all volatiles. Among the other chemical classes, alcohols, aldehydes and acids were the most represented with a percentage close to 10% for each one. 3-Butenyl isothiocyanate (43.10%), dimethyl trisulphide (10.66%) and 1-dodecanol (7.61%) were the compounds quantitatively most represented.

These results are quite different from those reported in our previous study on the volatiles of *B. incana* leaves (Tripodi et al. 2012); this can be explained considering that previously the SPME extraction technique was directly applied to the fresh plant leaves, and the characteristic "green leaf" volatiles, such as (E)-2-hexanal (leaf aldehyde), (Z)-3-hexenol (leaf alcohol) and, in general, C6 aldehydes and alcohols, resulted the main constituents of the leaf headspace; instead isothiocyanates were the main volatiles of the hydroalcoholic extract of *B. incana* leaves. Isothiocyanates arise from the glucosinolate hydrolysis after plant cell rupture, and in case of the hydroalcoholic extract, the procedure for sample preparation certainly favoured their formation (Fenwick et al. 1983). However, in both cases the class of isothio-cyanates.

The volatile fraction of the flowering top extract was composed mostly of sulphur compounds and nitriles. These two classes of compounds constituted about the 82% of the whole volatile fraction. The main constituents were dimethyl trisulphide (36.22%), dimethyl disulphide (18.51%), 3-methyl-3-butenenitrile (16.06%) and dimethyl tetrasulfide (8.16%). Isobutyl isothiocyanate ate and 3-butenyl isothiocyanate were the only isothiocyanates detected but they were present at very low levels representing only the 0.08% and 0.57% of the whole volatile fraction, respectively. The remaining compounds were present as minor constituents (<1%), except for 1-penten-3-one (1.95%), hexahydrofarnesyl acetone (1.32%), 1-octen-3-ol (1.07%), 1-octanol (1.11%) and limonene (2.20%).

The volatile profiles of the *B. incana* extracts showed significant differences. In particular, among sulphur compounds, isothiocyanates prevailed in the leaf extract, whereas sulphides in the flowering top one; similarly, Robertson et al. (1993) analyzing five different varieties of *Brassica napus* found that organic sulphides were among the major volatile compounds released from the flowers, whereas no isothiocyanates were detected.

Moreover, the headspace of the flowering top extract was very rich in nitriles while aldehydes, alcohols and acids were quantitatively less represented than in the leaf extract headspace.

Like isothiocyanates, nitriles are hydrolysis products of glucosinolates by the action of the myrosinase. The enzymatic cleavage can lead to different products depending on the glucosinolate structure and the presence of factors which modify the action of the enzyme. It has been demonstrated that ferrous ions and acidic conditions favour nitrile formation; moreover, nitriles are also favoured by the aglycone autolysis (Fenwick et al. 1983).

Bioactivity of B. incana extracts

Brassica incana extracts were able to inhibit pancreatic lipase and α -glucosidase in a dose-dependent manner, as reported in Figure 1. Although the observed inhibitions were not superior to the drug references used in the bioassays, orlistat and acarbose, it is the first time that these activities are reported for this plant species. In the case of pancreatic lipase, the IC₅₀ value was lower for the flowering top extract whereas in the glucosidase assay the best results were obtained for the leaves extract (Table 3). Both enzymes, pancreatic lipase and α -glucosidase, are key physiological and pharmacological targets for the treatment and prevention of metabolic disorders such as obesity, diabetes or the metabolic syndrome and therefore widely studied as pharmacological targets for phytochemicals (Ahmad et al. 2020; El-Nashar et al. 2021). Cruciferous plants (Brassicaceae family) have been presented several times as healthy food plants due to their content in bioactive compounds; particularly because they are rich in glucosinolates and their derived volatile sulphur compounds known as isothiocyanates, which are in relation with the prevention of certain cancers and disorders such as the metabolic syndrome (Esteve 2020; Melim et al. 2022). Although is it not clear in the literature that isothiocyanates act as pancreatic lipase or alpha-glucosidase inhibitors, other cruciferous plants have also demonstrated this kind of in vitro activity (Taviano et al. 2020). In previous work, several polyphenols, such as phenolic acids or flavonoids, have been detected in both B. incana leaf and flowering top extracts utilized in this study (Miceli et al. 2020). It was reported that extracts rich in polyphenols have a great capacity to inhibit enzymes involved in glucose and fat metabolism, such as alpha-glucosidase and lipase (Les et al. 2020). Thus, it can be assumed that the polyphenolic compounds are involved in the lipase or glucosidase inhibiting activity highlighted for B. incana extracts.

In relation with antiobesogenic and antidiabetic activity, these extracts have also revealed AGEs inhibitory properties. The

Table 1.	Composition	as volatile	constituents an	d classes o	f substances	of B.	incana	leaf h	ydroalcoholic	extrac
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Compound	LRI ^a on DB-5ms	LRI ^a on VF-WAXms	Amount ^b	Percentage
Sulphur compounds				
Dimethyl disulphide	742	1080	3959	1.99
Isobutyl isothiocyanate	929	1322	610	0.31
Dimethyl trisulphide	969	1388	21,163	10.66
3-Butenyl isothiocyanate	973	1462	85,532	43.10
Methyl methylthiomethyl disulphide	1135	1665	520	0.26
All			111,784	56.33
Nitriles				
4-Pentenenitrile	745	1279	5254	2.65
Heptanenitrile	991	1408	120	0.06
All			5374	2.71
Aldehydes				
(E)-2-Pentenal	/58	1131	38/	0.19
Heptanal	904	1186	//6	0.39
(E)-2-Heptenal	958	1329	5/1	0.29
Uctanal (7) 2 Octobel	1004	1422	1116	0.56
(E)-2-Octenal	1058	1433	140	0.07
Nonana	1105	1590	4201	2.12
Undecanal	1207	1501	7440	5./5
Dodocanal	1408	1711	1174	0.12
Tridecanal	1510	1817	559	0.39
Tetradecanal	1610	1972	1058	0.20
B-Cyclocitral	1220	1624	1388	0.55
Safranal	1200	1649	1071	0.54
All			20,128	10.14
Ketones			,	
6-Methyl-5-hepten-2-one	985	1340	249	0.13
All			249	0.13
Alcohols				
1-Hexanol	870	1347	188	0.09
1-Octen-3-ol	980	1448	233	0.12
2-Ethyl-1-hexanol	1028	1489	484	0.24
(<i>Z</i>)-2-Octen-1-ol	1060	1620	356	0.18
(E)-2-Octen-1-ol	1068	1616	101	0.05
1-Octanol	1071	1557	171	0.09
1-Nonanol	1173	1659	363	0.18
Dodecanol	1475	1966	15,106	7.61
Tetradecanol	1576	2171	5164	2.6
All			22,165	11.17
Acids	770	1626	507/	2.66
	//9	1051	52/6	2.66
Hexanoic acid	9/8	1851	/856	3.96
	11/1	2064	3/0/	1.8/
	1960	3000	2420	0.71
All			19,204	9.71
3-Methyl-1-hutyl acetate	876	1121	870	0.42
Hervi acetate	1011	1270	3001	1 51
1-Methylbutyl butanoate	1015	12/0	509	0.26
Butyl bexanoate	1185	1412	664	0.33
Hexyl hutanoate	1194	1415	228	0.11
Methyl hexadecanoate	1926	2216	3268	1.65
All	.,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		8498	4.28
Terpenes				
Limonene	1029	1193	4363	2.20
(Z)-Calamenene	1531	1835	1335	0.67
Guaiol	1597	2087	2333	1.18
Bulnesol	1667	2208	2391	1.20
All			10,423	5.25

^aLinear retention indexes calculated according to the Van Den Dool and Kratz equation.

^bPeak area arbitrary scale.

inhibitory activity is also better for the leaves than for the flowering top extracts (Figure 2); the level of fluorescence of BSA alone, and BSA + fructose, and BSA + fructose + treatments is included as Supplementary material. AGEs production is implicated in these metabolic diseases because of bad hyperglycaemia control; elevated glucose blood concentration leads to increased protein glycation generating a proinflammatory state (Garay-Sevilla et al. 2021). Nevertheless, hyperglycaemia not only contributes to AGEs production but also to oxidative stress and free radical release, inducing cellular ageing and disfunction (Silveira Rossi et al. 2022). For this reason, it is also important that α -glucosidase inhibitors may also act as anti-AGEs and antioxidant agents. Figure 3 shows the activity of leaf and flowering top extracts of *B. incana* against superoxide radicals in the xanthine/xanthine oxidase system. As it can be observed, the antiradical activity is

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Table 2.	Composition	as volatile	constituents	and classes	of substances	of B.	incana	flowerina to	p hvdroalcoholig	extract

Subput compounds 72 1080 32.307 18.96 Ionnethy divelphide 742 1080 32.307 18.96 Ionnethy divelphide 929 1328 63.208 36.62 3-bitterny isothiocyanate 973 1462 995 0.57 Dimethy divelphide 1216 1750 14.244 8.16 All 12.673 64.00 12.673 64.00 3-Methyl-3-buttenentinie 797 - 28.026 16.060 Berxyn Intrine 1336 1893 1442 683 Berxyn Intrine 1307 - 1028 6.39 All 0 085 633 039 Hexatal 803 1085 643 039 Hexatal 954 1166 100 0.05 Hexatal 954 1165 130 0.66 Hexatal 1004 1290 400 0.23 Hexatal 1004 1290 400 0.23 <th>Compounds</th> <th>LRI^a on DB-5ms</th> <th>LRI^a on VF-WAXms</th> <th>Amount^b</th> <th>Percentage</th>	Compounds	LRI ^a on DB-5ms	LRI ^a on VF-WAXms	Amount ^b	Percentage
Dimetry disulphide742188032.0718.96Dimetry disulphide969138237.30.08Dimetry trisulphide969138663.20836.22Jottary disubscyanate97.314.6299.50.57Dimetry trisulphide121617.9014.2448.16All112.617.9014.2448.81Nirtles7.9-28.02616.00Benzenerporpanetritie12.372.0411190.07Hindei-3-acetonitritie1307-10.280.59Hexanal803108.568.30.39Hesanal803108.568.30.39Hesanal9813.9010.190.07Benzelexproponentritie92.812.990.1710.00Benzelexproponentritie92.812.990.013.05Hesanal10015.8912.900.021.15Corbal10015.9913.360.201.15Benzilderbyde92.613.000.230.17Benzilderbyde92.613.000.230.15Octanal100015.9913.600.20Directhyl S-hepterb-2one98.513.402.510.16Benzilderbyde92.913.001.560.57All17.910.003.651.500.60Octan12.0715.013.660.201.14I -Perterb-3one19.8513.6	Sulphur compounds				
sobupit9291221370.08Joherhy fixuphide999138863.2636.263-Butery isothiocyanate97314629950.57Dimethy trausulide1216175014.248.16All1267189314220.803-Methyl-3-butenenitrile135189314420.83Benzyl nitrile135189314420.83Benzyl nitrile1807-10280.57All189314620.830.57All1807-10280.57All180310856630.39Heptanal90411861100.06(f-) z-Arteptadienal90411861100.06(f-) z-Arteptadienal90411861100.06(f-) z-Arteptadienal100013089.20.35Octanal100412094000.230.19Nomanal10513560.160.640.19Octanal104416453360.190.19Octanal1007130611550.660.830.32All120713002510.140.440.450.360.20Octanal1004164513560.350.190.350.20All1005135613500.660.350.350.20All1005135613500.660.350.3	Dimethyl disulphide	742	1080	32,307	18.96
Dimethy trisulphide969138863.20836.62Jattery is toticocyanate97314629956.57Dimethy tetrasulfide1216175014.2448.16Nirtles112.6732.441.08112.6736.4.00Bernzen reproparentiritie133712.4411130.037I H-Indole-3-acetonitritie1307-10.220.59All1136189314420.830.850.39Hardole-3-acetonitritie80318851000.050.39All80318851000.050.170.170.17Bernzenerproparentiritie95813392.990.170.17Bernziderbyde95813302.310.130.130.150.06(C) Lapterbal95813301.150.060.230.130.150.050.160.050.160.020.170.150.060.220.170.150.060.220.170.150.060.150.050.150.050.150.050.150.050.150.050.150.050.150.050.150.050.150.050.150.050.150.060.220.130.150.060.220.130.140.140.150.160.020.150.140.140.150.140.140.150.140.150.160.050.150.160.15 <td>Isobutyl isothiocyanate</td> <td>929</td> <td>1322</td> <td>137</td> <td>0.08</td>	Isobutyl isothiocyanate	929	1322	137	0.08
3-biterly listinicyanate 973 1462 995 0.57 All 112,073 64.40 112,073 64.40 3-Methyl-3-butenenttrile 759 - 28,025 66.06 Benzyl intrile 136 1893 1442 0.83 Benzyl intrile 1237 20.41 119 0.07 All 1237 20.41 119 0.07 All 30487 7.54 30,485 7.54 All 105 68.3 0.39 110 0.06 Ithinole-scottonitile 904 1186 110 0.06 Ithinole-scottonitile 903 1126 10 0.06 Ithinole-scottonitile 904 1186 10 0.06 Ithinole-scottonitile 903 1203 201 0.13 Ithinole-scottonitile 903 1203 10.15 0.05 Ithinole-scottonitile 1004 1206 10.5 0.06 Ithinole-scottonitile 1	Dimethyl trisulphide	969	1388	63,208	36.62
Dimethy tetrasultice 126 175 14,244 8,16 Nitries 112,673 64.40 Nitries - 28,026 (6.66) Benzyn proprenentile 136 1933 1442 0.83 Benzeneproprenentile 137 2041 119 0.07 Hindole-3-actontrile 1807 - 1028 0.59 Ald 803 1085 683 0.39 Heptanal 803 1085 683 0.39 Heptanal 904 1186 110 0.06 Cicl-2-Heptanal 992 0.17 19 19 0.03 Cicl-2-Heptanal 904 1186 100 0.06 0.63 0.39 PhenylaceLidehyde 1004 1240 400 0.23 0.03 0.01 0.05 0.02 0.03 0.02 0.03 0.01 0.05 0.02 0.03 0.02 0.01 0.05 0.02 0.05 0.01 0.05 0	3-Butenyl isothiocyanate	973	1462	995	0.57
All TL2D13 H2D13 H2D13 H2D13 H2D13 H2D13 H2D13 3-Methyl-3-butenenttrile 1326 1893 1442 0.83 Benzon intrile 1127 20/41 119 0.07 Benzon intrile 1237 20/41 119 0.07 1028 0.59 All 1237 20/41 119 0.07 1028 0.59 All 1237 20/41 119 0.07 1028 0.59 All 100 1186 110 0.066 0.67 1186 100 0.066 (E/2)-4Heptanal 958 1339 239 0.03 0.03 0.05 0.133 0.13 0.056 0.023 Phenylacetaldehyde 962 1350 0.666 0.23 Phenylacetaldehyde 1000 1505 0.666 0.23 0.056 0.056 0.056 0.056 0.056 0.056 0.056 0.056 0.056 0.056 0.056 0.056 0.056 0.056 <td>Dimethyl tetrasulfide</td> <td>1216</td> <td>1750</td> <td>14,244</td> <td>8.16</td>	Dimethyl tetrasulfide	1216	1750	14,244	8.16
minutes 759 - 28,025 16,066 Benzy inititie 136 1893 1442 0.03 Benzy inititie 1367 2041 119 0.07 Hi-Indole-3-acetonitrile 1307 - 1028 0.39 Hatsinal 1307 - 1028 0.39 Hexanal 904 1186 110 0.06 Hexanal 904 1186 110 0.06 (f) 2-Meptranal 598 1329 291 0.117 Benzandlehyde 904 1368 190 0.06 Octanal 1000 1368 92 0.035 Octanal 1004 1290 400 0.23 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1005 1360 2.00 1.05 All 1027 1501 356 2.02 2.02 All 1105 1340 2.51 0.141	All			112,673	64.40
brack 126 1263 1264 0.033 Benzyl nitrik 1136 1893 1442 0.037 Benzyl nitrik 1237 2041 113 0.037 All - 1028 0.59 All - 30,495 1754 All - 30,495 0.37 All 904 1186 110 0.060 (f)-2+Atteptanal 904 1290 400 0.233 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1105 1396 1150 0.66 Decanal 1207 120 3405 1.55 6-Methyl-5-heptadien-2-one 985 1340 251 0.14 6-Methyl-5-heptadien-2-one 1986	3-Methyl-3-butenenitrile	750	_	28.026	16.06
Berzeneprosenentinile 1237 2041 119 007 1H-indole-3-acetonitrile 1807 - 1028 0.59 Ald 803 1085 683 0.99 Hexanal 803 1085 683 0.99 Hetpanal 904 1186 110 0.06 (F)-2-Heptanal 992 1530 231 0.13 (E,F)-2-Heptanal 1000 1508 92 0.05 Octanal 1004 1290 400 0.23 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1007 1501 356 0.20 All 251 0.14 6.40 0.23 Nonanal 105 1396 1150 0.66 0.20 All 252 0.20 1.32 0.30 1.32 All 167 152 1945 1.11 1-Octen-3-ol 985 1340 </td <td>Benzyl nitrile</td> <td>1136</td> <td>1893</td> <td>1442</td> <td>0.83</td>	Benzyl nitrile	1136	1893	1442	0.83
1H-Incide-3-acetonitrile 1807 - 1028 0.99 All 30,495 75.44 Aldehydes - 30,495 683 0.39 Hexnal 803 1085 683 0.39 Hexnal 994 1186 110 0.06 (C)-2-Heptenal 998 1329 299 0.17 Benzaldehyde 988 1329 299 0.17 Benzaldehyde 1004 1290 400 0.23 Ctanal 1004 1290 400 0.23 Nonand 1105 1396 1150 0.66 Decanal 1207 1501 356 0.20 All 1207 1501 356 0.20 All 1207 1501 356 0.20 All 1207 1340 251 0.14 6-Methyl-5-hepten-2-one 985 1340 251 0.14 6-Methyl-5-heptadeanone (hexahydrofarnesyl acetone) 1964	Benzenepropanenitrile	1237	2041	119	0.07
All 30,495 30,495 75,4 Heynanal 803 1085 683 0.39 Heynanal 904 1186 1100 0.06 (E)-2-Heptenal 958 1329 209 0.17 Benzaldehyde 962 1330 231 0.03 (E)-2-Heptenal 1000 1508 9.2 0.05 Octanal 1000 1508 9.2 0.05 Octanal 1004 1290 400 0.23 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1105 1396 1150 0.66 Decanal 1207 1501 366 0.20 All 1645 1380 0.23 0.30 All 1647 1366 0.20 0.05 All 1627 196 1515 0.14 Octanal 1007 1507 332 0.30 6A10-Trimehyl-2-pentadecanone (hexahydrofarneyl acetone) 196 1582 332 0.30 6A10-Trimehyl-2-pentadecanone (hexahydrofarneyl acetone) 196 1649 107 (C)-2-Oten-1-ol 1068 1667 750 107 (C)-2-Oten-1-ol	1H-Indole-3-acetonitrile	1807	_	1028	0.59
Aldehyde's Hexnanl (2005) Heptanal (2005) (2)-2-Heptnanl 964 1186 110 006 (2)-2-Heptnanl 958 1329 299 017 Benzaldehyde 962 1530 231 0.13 (<i>E</i> , 2)-2, 4-Heptadienal 0000 1508 92 0.03 Octanal 0000 1508 92 0.03 Phenylacetaddehyde 1044 1645 3.36 0.19 Nonanal 1105 1396 1150 0.66 Decanal 1207 1501 3.56 0.20 All	All			30,495	17.54
Hexanal 803 1085 683 0.39 Heptanal 904 1166 110 0.06 (E)-2-Heptenal 958 1329 299 0.17 Benzaldehyde 962 1530 231 0.13 (E)-2-4-Heptadienal 1000 1508 92 0.05 Octanal 1004 1200 400 0.23 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1105 1396 1150 0.66 Decanal 1207 1501 356 0.20 All 3657 2.10 Ketones 1 1020 3405 1.95 6-Methyl-5-hepten2-one 921 1340 251 0.14 6-Methyl-5-hepten2-one 1096 1582 532 0.30 6-Methyl-5-hepten2-one 1980 1448 1871 1.07 (f)-2-coten-1-ol 1068 1616 867 0.50 All 173 1659 1945 1.11 1-Octen-3-ol 1068 1616 867 0.50 All 173 1659 1946 1.11 1-Octen-1-ol 1068 <td>Aldehydes</td> <td></td> <td></td> <td></td> <td></td>	Aldehydes				
Heptanal 994 1186 110 0.06 (f)2-Heptanal 958 1329 299 0.17 Benzaldehyde 962 1530 231 0.13 (f)2-Heptadianal 1000 1508 92 0.05 Octanal 1004 1290 400 0.23 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1005 1396 1150 0.66 Decanal 1207 1501 356 0.20 All 1207 1020 3405 1.55 6-Methyl-5-heptaclen-2-one 985 1340 251 0.44 6-Methyl-5-heptaclen-2-one 985 1340 251 0.30 6-All OrTimethyl-2-pentadecanone (hexahydrofarnesyl acetone) 1844 2119 2302 132 All 6490 322 1.02 1.07 1.07 1.07 1.07 1.07 (f)2-2-Oten-1-ol 1068 1616 867 0.50	Hexanal	803	1085	683	0.39
(b) 2-14-ptential 958 1329 299 0.17 Benzaldehyde 962 1530 231 0.13 (b) 2-24-Heptadienal 1000 1508 92 0.05 Octanal 1004 1290 400 0.232 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1105 1396 1150 0.66 Decanal 1207 1501 356 0.20 All	Heptanal	904	1186	110	0.06
benzaldehyde 992 1540 251 0.13 Cf.2)-2.4 Heptadienal 1000 1508 92 0.05 Octanal 1004 1290 400 0.23 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1105 1396 1150 0.66 Decanal 1207 1501 3567 2.10 Ketones	(E)-2-Heptenal	958	1329	299	0.17
(b.2)-24-Heptadienal 1000 1508 92 0.05 Octanal 1004 1290 400 0.23 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1105 1396 1150 0.66 Decanal 1207 1501 356 0.20 All 3657 2.10 Ketones 1 1202 3405 1.95 6-Methyl-5.5-heptadien-2-one 985 1340 251 0.14 6-Alt Dirfmethyl-2-pentadecanone (hexahydrofarnesyl acetone) 1844 2119 2302 1.32 Ali 6430 3.72 4100 3657 0.50 1.12 Alcohols 6490 3.72 410 6490 3.72 Alcohol 1071 1557 1945 1.11 1.07 1.02 0.50 1.11 1-Octanol 1071 1557 1945 1.11 1.90 65 0.50 1.11 </td <td>Benzaldehyde</td> <td>962</td> <td>1530</td> <td>231</td> <td>0.13</td>	Benzaldehyde	962	1530	231	0.13
Octanal 1004 1290 400 0.23 Phenylacetaldehyde 1044 1645 336 0.19 Nonanal 1105 1396 1150 0.66 Decanal 1207 1501 336 0.20 All 3657 2.10 Ketones 721 1020 3405 1.95 6-Methyl-5-hepten-2-one 985 1340 251 0.14 6-Methyl-5-hepten-2-one 985 1340 251 0.14 6-Methyl-5-hepten-2-one 1096 1582 532 0.30 All 2119 2.302 1.32 All 2119 2.302 1.32 All 1.07 1.557 1.945 1.11 1-Octen-3-01 1068 1616 867 0.50 1-Octen-1-01 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1687 874	(E.E)-2.4-Heptadienai	1000	1508	92	0.05
Interplace 1044 1043 1320 0.13 Nonanal 1207 1501 356 0.20 All 207 1501 356 0.20 Ketones - - 3657 2.10 1-Penten-3-one 721 1020 3405 1.95 6-Methyl-5-hepten-2-one 985 1340 251 0.14 6-Methyl-3-5-heptadien-2-one 1096 1582 532 0.30 6.10-Trimethyl-2-pentadecanone (hexahydrofarnesyl acetone) 1844 2119 2302 1.32 All - - 6490 3.72 1.07 1-Octen-3-ol 980 1448 1871 1.07 1-Octan-3-ol 1068 1616 867 0.50 All - - 4781 2.74 Acids - 4781 2.74 2-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 996 - 315 <td< td=""><td>Ocidiidi Dhanvlacataldahvda</td><td>1004</td><td>1290</td><td>400</td><td>0.25</td></td<>	Ocidiidi Dhanvlacataldahvda	1004	1290	400	0.25
Institution 1103 1203 1205 1205 0.000 All 3657 2.10 Ketones - 3657 2.10 1-Penten-3-one 721 1020 3405 1.95 6-Methyl-5-hepten-2-one 985 1340 251 0.14 6-Methyl-5-hepten-2-one 1096 1582 532 0.30 6.4.10-Trimethyl-2-pentadecanone (hexahydrofarnesyl acetone) 1844 2119 2302 1.32 All 640 572 0.50 3.22 All 6490 3.72 Alcohois - - 6490 3.72 1.11 1.07 1.557 1945 1.11 1-Octanol 1068 1616 867 0.50 1.11 1-Nonanol 1173 1659 98 0.06 All 4781 2.74 420 2.44 Acids 1687 874 0.50 2.74 2-Methyl-heptenoic acid 996 - 315<	Nonanal	1044	1396	1150	0.19
All 365 2.0 Ketones	Decanal	1207	1501	356	0.00
Ketones International stress Internaternational stress International str	All	1207	1301	3657	2.10
1-Penten-3-one 721 1020 3405 1.95 6-Methyl-5-hepten2-one 985 1340 251 0.14 6-Methyl-5-heptendien-2-one 1096 1582 322 0.30 6.4.10-Trimethyl-2-pentadecanone (hexahydrofarnesyl acetone) 1844 2119 2302 1.32 All 6490 3.72 Alcohois - 6490 3.72 1-Octen-3-ol 980 1448 1871 1.07 (6-2-Octen-1-ol 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All - 4781 2.74 Acids - 4781 0.50 2-Methylbutanoic acid 834 1681 417 0.24 2-Methyl-t-pentenoic acid 996 - 315 0.18 2-Hethyl-t-pentonic acid 1115 1129 165 0.99 Octanoic acid 1365 266 3.36 0.48 Decanoic acid 13	Ketones			5007	200
6-Methyl-5-hepten-2-one 985 1340 251 0.14 6-Methyl-5.5-heptadien-2-one 1096 1582 532 0.30 6-Authyl-5.5-heptadien-2-one 1086 1582 532 0.30 6-Auto-Trimethyl-2-pentadecanone (hexahydrofarnesyl acetone) 1844 2119 2302 1.32 All 6410 70 6490 3.72 Alcohols 1071 1557 1945 1.11 1-Octenol 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All 4781 2.74 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4 2.4	1-Penten-3-one	721	1020	3405	1.95
6-Methyl-3.5-heptadien-2-one 1096 1582 532 0.30 6.4.10-Trimethyl-2-pentadecanone (hexahydrofarnesyl acetone) 1844 2119 2302 1.32 All 6490 3.72 6490 3.72 Alcohols - 6490 3.72 1-Octen-3-ol 980 1448 1871 1.07 (C)- 2-Octen-1-ol 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All 41 4781 2.74 Acids 41 417 0.24 All 845 1687 874 0.50 (2)-3-Hexenoic acid 992 1940 80 0.05 2-Methylbutanoic acid 115 1129 165 0.09 Octanoic acid 115 1129 165 0.38 2-Methyl-L-pentencic acid 1268 2165 8.36 0.48 2	6-Methyl-5-hepten-2-one	985	1340	251	0.14
6.4.10-Trimethyl-2-pentadecanone (hexahydrofarnesyl acetone) 1844 2119 2302 1.32 All Allohols 6490 3.72 1-Octen-3-ol 980 1448 1871 1.07 (E)-2-Octen-1-ol 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All 4781 2.74 Acids 1681 417 0.24 2-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 992 1940 80 0.50 2-Methylbutanoic acid 996 - 315 0.18 2-Methyl-4-pentenoic acid 1171 1064 665 0.38 0-Ctanoic acid 1171 2064 665 0.38 0-Enonic acid 1165 1268 2165 836 0.48 Decanoic acid 1268 2165 836 0.48 Decanoic acid 1268 2165 836 0.48	6-Methyl-3.5-heptadien-2-one	1096	1582	532	0.30
All 6490 3.72 Alcohols 7 1-Octen-3-ol 980 1448 1871 1.07 (E)- 2-Octen-1-ol 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All 4781 2.74 Acids 4781 0.24 2-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 845 1687 874 0.50 2-Methylbutanoic acid 992 1940 80 0.05 2-Methylbutanoic acid 1115 1129 165 0.09 Octanoic acid 1155 1129 165 0.38 Nonanoic acid 1268 2165 836 0.42 All 274 3738 2.14 214 Esters 3738 2.14 254 0.15 Phenyl acetate 1056 1243 254 0.15 Phenyl acetate 1064 1660	6.4.10-Trimethyl-2-pentadecanone (hexahydrofarnesyl acetone)	1844	2119	2302	1.32
Alcohols 1-Octen-3-ol 980 1448 1871 1.07 (F) - 2-Octen-1-ol 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All - 4781 2.74 Acids 1621 417 0.24 3-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 992 1940 80 0.05 2-Methylbutanoic acid 996 - 315 0.18 2-Methylbutanoic acid 996 - 315 0.18 2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Ethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1165 2267 386 0.22 All 3738 2.14 3738 2.14 Esters 3765 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 <t< td=""><td>All</td><td></td><td></td><td>6490</td><td>3.72</td></t<>	All			6490	3.72
1-Octen-3-ol 980 1448 1871 1.07 (E)- 2-Octen-1-ol 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All - 4781 2.74 Acids - 4781 2.74 3-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 992 1940 80 0.05 (Z)-3-Hexenoic acid 996 - 315 0.18 2-Methylb-4-pentenoic acid 996 - 315 0.18 2-Ethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Decanoic acid 1268 2165 836 0.48 Decanoic acid 1268 2267 386 0.22 All - 3738 2.14 Esters - 3738 2.14 Sters - 3738 2.14	Alcohols				
(b) 2-Octen-1-ol 1068 1616 867 0.50 1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All 4781 2.74 Acids 4781 2.74 3-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 845 1687 874 0.50 (Z)-3-Hexenoic acid 992 1940 80 0.05 2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Ethylhexanoic acid 1171 2064 665 0.38 Nonanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All 3738 2.11 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Octaroet 1314 2071<	1-Octen-3-ol	980	1448	1871	1.07
1-Octanol 1071 1557 1945 1.11 1-Nonanol 1173 1659 98 0.06 All 4781 2.74 Acids 417 0.24 3-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 845 1687 874 0.50 (Z)-3-Hexenoic acid 992 1940 80 0.05 2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Ethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.42 All 3738 2.14 2.14 290 0.17 Petryl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1064 1660 86 0.05 Methyl octanoate	(E)- 2-Octen-1-ol	1068	1616	867	0.50
1173 1659 96 0.06 All 4781 2.74 Acids 1173 1681 417 0.24 3-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 845 1687 874 0.50 (Z)-3-Hexenoic acid 996 - 315 0.18 2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Hethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All 3738 2.14 2165 836 0.21 Esters 3-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Carveyl acetate 1314 2071 150 0.09		1071	1557	1945	1.11
Arili 4761 2.74 Acids 1 4761 2.74 3-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 845 1687 874 0.50 (Z)-3-Hexenoic acid 992 1940 80 0.05 2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Ethylbexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All 3-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05		11/5	1059	90 //781	0.00
3-Methylbutanoic acid 834 1681 417 0.24 2-Methylbutanoic acid 845 1687 874 0.50 (Z)-3-Hexenoic acid 992 1940 80 0.05 2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Ethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All 3-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05 Methyl 0-thylpropanoate 1347 1547 89 0.05	Arids			4701	2.74
2-Methylbutanoic acid 831 1687 187 0.51 2-Methylbutanoic acid 845 1687 874 0.50 (Z)-3-Hexenoic acid 992 1940 80 0.05 2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Ethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All - - 3738 2.14 Esters - - 3738 2.14 S-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	3-Methylbutanoic acid	834	1681	417	0.24
(Z)-3-Hexenoic acid 992 1940 80 0.05 2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Ethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All - 3738 2.14 Esters - 3738 2.14 S-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	2-Methylbutanoic acid	845	1687	874	0.50
2-Methyl-4-pentenoic acid 996 - 315 0.18 2-Ethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All - 3738 2.14 Esters - 3738 2.14 S-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	(Z)-3-Hexenoic acid	992	1940	80	0.05
2-Ethylhexanoic acid 1115 1129 165 0.09 Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All	2-Methyl-4-pentenoic acid	996	_	315	0.18
Octanoic acid 1171 2064 665 0.38 Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All - 3738 2.14 Esters - 3738 2.14 3-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	2-Ethylhexanoic acid	1115	1129	165	0.09
Nonanoic acid 1268 2165 836 0.48 Decanoic acid 1365 2267 386 0.22 All 3738 2.14 Esters 3 3 2.14 3-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	Octanoic acid	1171	2064	665	0.38
Decanoic acid 1365 2267 386 0.22 All 3738 2.14 Esters 3 7 290 0.17 3-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	Nonanoic acid	1268	2165	836	0.48
All 3738 2.14 Esters 3-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	Decanoic acid	1365	2267	386	0.22
Esters 3-Methyl-1-butyl acetate 876 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	All			3738	2.14
3-Methyl-1-butyl acetate 8/6 1121 290 0.17 Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	Esters	076	1121	200	0.17
Pentyl 2-methylpropanoate 1056 1243 254 0.15 Phenyl acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	3-Methyl-1-butyl acetate	8/6	1121	290	0.17
Printipil acetate 1064 1660 86 0.05 Methyl octanoate 1127 1411 1162 0.67 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05	Pentyl 2-methylpropanoate	1050	1243	254	0.15
Methyl octatione 1127 1411 1102 0.07 Carveyl acetate 1314 2071 150 0.09 Octyl 2-methylpropanoate 1347 1547 89 0.05 Darweyl acetate 1322 1527 151 0.57	Methyl octanoate	1004	1000	00 1162	0.05
Octyl 2-methylpropanoate 1347 1547 89 0.05 Darwid 2-methylpropanoate 1322 1577 89 0.05	Carveyl acetate	1314	2071	1102	0.07
	Octyl 2-methylpropapoate	1314	1547	89	0.05
penzyi 3-meuryiputanoate 1392 1852 121 0.07	Benzyl 3-methylbutanoate	1392	1852	121	0.07
1-Methylethyl decanoate 1428 1615 409 0.23	1-Methylethyl decanoate	1428	1615	409	0.23
1-Octen-3-yl hexanoate 1507 – 328 0.19	1-Octen-3-yl hexanoate	1507	_	328	0.19
1-Methylethyl tetradecanoate 1826 2017 57 0.03	1-Methylethyl tetradecanoate	1826	2017	57	0.03
Methyl hexadecanoate 1926 2216 1563 0.90	Methyl hexadecanoate	1926	2216	1563	0.90
Methyl linoleate 2095 2480 76 0.04	Methyl linoleate	2095	2480	76	0.04
Methyl linolenate 2101 2503 357 0.20	Methyl linolenate	2101	2503	357	0.20
All 4790 2.83	All			4790	2.83
Terpenes	Terpenes		1005		
α-rinene 933 1025 149 0.09 0 Dimension 0.70 1100 0.02 0.10	α-rinene	933	1025	149	0.09
p-rinene 9/8 1108 333 0.19	p-rinene	9/8 1025	1108	333	0.19
Provincine 1025 12/0 600 0.34		1025	12/0	000	0.34 2.20
Linuicie 1029 1195 3032 2.20 Fucalvatol 1032 1206 715 0.41	Europene	1029	1706	2032 715	2.20
receivation 1000 1200 710 0.41 a-konharane 1174 1621 205 0.46	a-konhorone	1174	1671	213 805	0.41
Safranal 124 1021 000 0.40	Safranal	1200	1639	451	0.40
B-Cyclocitral 1220 1623 530 0.30	β-Cvclocitral	1220	1623	530	0.30
Citronellol 1232 1757 68 0.04	Citronellol	1232	1757	68	0.04
Carvacrol 1299 2225 110 0.06	Carvacrol	1299	2225	110	0.06
Orivone 1354 – 133 0.08	Orivone	1354	-	133	0.08

(continued)

Table 2. Continued.				
Compounds	LRI ^a on DB-5ms	LRI ^a on VF-WAXms	Amount ^b	Percentage
All			7740	4.43
Hydrocarbons				
Heptadecane	1700	1700	59	0.03
Octadecane	1800	1800	40	0.02
1-Eicosene	1994	2051	69	0.04
All			168	0.10

*Linear retention indexes calculated according to the Van Den Dool and Kratz equation.

^bPeak area arbitrary scale.



Figure 1. Inhibition of pancreatic lipase (A) and α -glucosidase (B) by *B. incana* leaf and flowering top hydroalcoholic extracts. Orlistat and acarbose were used as positive control substances.

Table 3. IC ₅₀ values for <i>B. incana</i> leaf and flowering top hydroalco	pholic extracts and drug compounds used as references.
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	IC ₅₀ values (mg/mL) in different bioassays						
Samples	AGEs	Superoxide	Glucosidase	Lipase			
B. incana (leaf)	0.192 ± 0.024^{a}	0.022 ± 0.003^{a}	0.968 ± 0.141^{a}	1.086 ± 0.319^{a}			
B. incana (flowering top)	0.262 ± 0.020^{a}	0.038 ± 0.012^{a}	1.921 ± 0.321^{a}	0.939 ± 0.131^{a}			
AMG	0.0744 ± 0.017^{b}	-	-	-			
Trolox	-	0.028 ± 0.001^{a}	-	-			
Acarbose	-	-	0.306 ± 0.039^{b}	-			
Orlistat	-	-	-	0.0277 ± 0.015^{b}			

Results are expressed as average \pm SEM of at least three independent experiments. ^{a,b} Different letters within the same column indicate significant differences between mean values (P < 0.05). No significant differences were found between leaves and flowering tops using ANOVA and Tukey for multiple comparison statistical analyses.

slightly better for the leaf extract, whose IC_{50} values are lower than the values obtained for the flowering top one (Table 3). The antioxidant activity of *B. incana* has already been published (Miceli et al. 2020; Picchi et al. 2020) but this is the first time that is performed against superoxide radicals generated by xanthine oxidase and compared with trolox using non-linear regression analysis. Our results are in accordance with the previous work made by the authors as the leaf extract is better as radical scavenger than the flowering top (Miceli et al. 2020); nevertheless, it is surprising that the capacity of our extracts to



Concentration (mcg/ml)

Figure 2. Inhibition of advanced glycation end-products (AGEs) by *B. incana* leaf and flowering top hydroalcoholic extracts (A) compared to aminoguanidine (B), used as positive control substance.



Figure 3. Antioxidant activity against superoxide radicals by B. incana leaf and flowering top hydroalcoholic extracts. Trolox was used as positive control substance.

inhibit superoxide radicals is even better than the activity displayed by Trolox (Figure 3 and Table 3). Previous work, as by Miceli et al. (2020), have also dealt with the presence of phenolics and have demonstrated the absence of toxicity against *A*. *salina* nauplii, which is also important to recommend a plant matrix as a healthy functional food. Considering that these extracts act as enzyme inhibitors of pancreatic lipase and α -glucosidase and as antioxidant and anti-AGEs agents, they could represent an interesting source of bioactive molecules.

Conclusions

Herein, the volatile profile and the antidiabetic and anti-obesity potential of leaves and flowering tops from *Brassica incana* grown wild in Sicily (Italy) are reported. Significant differences in the volatile composition of the leaf and flowering top hydroalcoholic extracts have been highlighted. In particular, among sulphur compounds, isothiocyanates prevailed in the former, being 3-butenyl isothiocyanate the main component. Both extracts have been shown for the first time to inhibit pancreatic lipase, α -glucosidase, advanced glycation end-products and superoxide radicals in the xanthine/xanthine oxidase system, although the flowering top extract displayed better pancreatic lipase inhibiting activity, there were not significant differences between the leaf extract and the flowering top. The present findings indicate that leaves and flowering tops from *B. incana* are a source of functional ingredients that could act as antidiabetic and anti-obeso-genic agents.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Maria Fernanda Taviano (b) http://orcid.org/0000-0002-4314-5598 Concetta Condurso (b) http://orcid.org/0000-0003-2799-7712 Antonella Verzera (b) http://orcid.org/0000-0002-6162-9297 Maria Merlino (b) http://orcid.org/0000-0003-3888-1811 Natalizia Miceli (b) http://orcid.org/0000-0002-1611-6564

References

- Ahmad B, Friar EP, Vohra MS, Garrett MD, Serpell CJ, Fong IL, Wong EH. 2020. Mechanisms of action for the anti-obesogenic activities of phytochemicals. Phytochemistry. 180:112513.
- Carullo G, Cappello AR, Frattaruolo L, Badolato M, Armentano B, Aiello F. 2017. Quercetin and derivatives: useful tools in inflammation and pain management. Future Med Chem. 9(1):79–93.
- Cincotta F, Verzera A, Tripodi G, Condurso C. 2018. Non-intentionally added substances in PET bottled mineral water during the shelf-life. Eur Food Res Technol. 244(3):433-439.
- El-Nashar HAS, Mostafa NM, El-Shazly M, Eldahshan OA. 2021. The role of plant-derived compounds in managing diabetes mellitus: a review of literature from 2014 To 2019. Curr Med Chem. 28(23):4694–4730.
- Esteve M. 2020. Mechanisms underlying biological effects of cruciferous glucosinolate-derived isothiocyanates/indoles: a focus on metabolic syndrome. Front Nutr. 7:111.
- Fenwick GR, Heaney RK, Mullin WJ, VanEtten CH. 1983. Glucosinolates and their breakdown products in food and food plants. Crit Rev Food Sci Nutr. 18(2):123–201.
- Garay-Sevilla ME, Rojas A, Portero-Otin M, Uribarri J. 2021. Dietary AGEs as exogenous boosters of inflammation. Nutrients. 13(8):2802.
- Heaney RK, Fenwick GR, Mithen RF, Lewis BG. 1987. Glucosinolates of wild and cultivated *Brassica* species. Phytochemistry. 26(7):1969–1973.

- Heywood VH. 1964. Brassica L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, editors. Flora Europaea. Cambridge: Cambridge University Press; p. 335–339.
- Horn PJ, Vaughan JG. 1983. Seed glucosinolates of fourteen wild *Brassica* species. Phytochemistry. 22(2):465–470.
- Les F, Cásedas G, Valero MS, Arbonés-Mainar JM, López V. 2020. Rock tea (Jasonia glutinosa (L.) DC.) polyphenolic extract inhibits triglyceride accumulation in 3T3-L1 adipocyte-like cells and obesity related enzymes in vitro. Food Funct. 11(10):8931–8938.
- Liu S, Xiao P, Kuang Y, Hao J, Huang T, Liu E. 2021. Flavonoids from sea buckthorn: a review on phytochemistry, pharmacokinetics and role in metabolic diseases. J Food Biochem. 45(5):e13724.
- Marhold KB. 2011. Euro + Med Plantbase the Information Resource for Euro-Mediterranean Plant Diversity [accessed on 1 February 2020]. http:// www.emplantbase.org/home.html.
- Melim C, Lauro MR, Pires IM, Oliveira PJ, Cabral C. 2022. The role of glucosinolates from cruciferous vegetables (Brassicaceae) in gastrointestinal cancers: from prevention to therapeutics. Pharmaceutics. 14(1):190.
- Miceli N, Cavò E, Ragusa M, Cacciola F, Mondello L, Dugo L, Acquaviva R, Malfa GA, Marino A, D'Arrigo M, et al. 2020. *Brassica incana* Ten. (Brassicaceae): phenolic constituents, antioxidant and cytotoxic properties of the leaf and flowering top extracts. Molecules. 25(6):1461.
- Mustafa AM, Mazzara E, Abouelenein D, Angeloni S, Nunez S, Sagratini G, López V, Cespi M, Vittori S, Caprioli G, et al. 2022. Optimization of solvent-free microwave-assisted hydrodiffusion and gravity extraction of *Morus nigra* L. fruits maximizing polyphenols, sugar content, and biological activities using central composite design. Pharmaceuticals. 15(1):99.
- Nasri I, Chawech R, Girardi C, Mas E, Ferrand A, Vergnolle N, Fabre N, Mezghani-Jarraya R, Racaud-Sultan C. 2017. Anti-inflammatory and anticancer effects of flavonol glycosides from *Diplotaxis harra* through GSK3β regulation in intestinal cells. Pharm Biol. 55(1):124–131.
- Picchi V, Lo Scalzo R, Tava A, Doria F, Argento S, Toscano S, Treccarichi S, Branca F. 2020. Phytochemical characterization and *in vitro* antioxidant properties of four *Brassica* wild species from Italy. Molecules. 25(15):3495.
- Robertson GW, Griffiths DW, Smith WM, Butcher RD. 1993. The application of thermal desorption-gas chromatography-mass spectrometry to the analyses of flower volatiles from five varieties of oilseed rape (*Brassica napus* spp. *oleifera*. Phytochem Anal. 4(4):152–157.)
- Silveira Rossi JL, Barbalho SM, Reverete de Araujo R, Bechara MD, Sloan KP, Sloan LA. 2022. Metabolic syndrome and cardiovascular diseases: going beyond traditional risk factors. Diabetes Metab Res Rev. 38(3): e3502.
- Spínola V, Castilho PC. 2017. Evaluation of Asteraceae herbal extracts in the management of diabetes and obesity. Contribution of caffeoylquinic acids on the inhibition of digestive enzymes activity and formation of advanced glycation end-products (*in vitro*). Phytochemistry. 143:29–35.
- Sriramavaratharajan V, Murugan R. 2018. Cumin scented leaf essential oil of *Cinnamomum chemungianum*: compositions and their *in vitro* antioxidant, α-amylase, α-glucosidase and lipase inhibitory activities. Nat Prod Res. 32(17):2081–2084.
- Taviano MF, Miceli N, Acquaviva R, Malfa GA, Ragusa S, Giordano D, Cásedas G, Les F, López V. 2020. Cytotoxic, antioxidant, and enzyme inhibitory properties of the traditional medicinal plant *Matthiola incana* (L.) R. Br. Biology. 9(7):163.
- Tripodi G, Verzera A, Dima G, Condurso C, Ragusa S. 2012. Brassica fruticulosa Cyr. and Brassica incana Ten. (Brassicaceae) as Mediterranean traditional wild vegetables: a valuable source of bioactive compounds. J Essent Oil Res. 24(6):539–545.
- Velasco L, Becker HC. 2000. Variability for seed glucosinolates in a germplasm collection of the genus *Brassica*. Genet Resour Crop Evol. 47(3): 231–238.