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Mary Jenefer Sharmilan P and Dorothy Jaganathan

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BIOACTIVE COMPOUNDS IN CAULIFLOWER LEAVES (*BRASSICA OLERACEA VAR.BOTRYTIS*) USING GCMS

Mary Jenefer Sharmilan P[1] and Dorothy Jaganathan[2]

[1,2]Department of Food Service Management and Dietetics Avinashilingam Institute for Home Science and Higher Education for Women University Coimbatore 6410043

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ABSTRACT

Gas chromatography (GC) is a widely applied technique and has played a fundamental role in determining how many components and in what proportion they exist in a mixture. There is a great interest in plant and plant derived phytochemicals as food source. Cauliflower is one such popularly consumed vegetable, possessing potent bioactive components where the leaves of the vegetables are often neglected or discarded and used as fodder. Hence an attempt was made to analyze the presence of different compounds using GCMS there by exploring the potential benefits of these leaves which is solely ignored by majority of population especially in Tamil Nadu. The leaves were washed and oven dried at 40^0 C. The dried leaf were screened through GCMS and the results confirmed the presence of twenty eight different compounds such as linolenic acid an essential fatty acid, few other fatty acids namely palmitic acid, stearic acid, mysteric acids, phytol and stigmasterol and antioxidant and antimicrobial potential compounds. The present study throws light on the presence of promising compounds that can be consumed in regular diet in maintaining good health and can be technically applied in preparing natural therapeutic medicines to combat various degenerative diseases and can also be used in food processing industries due to its antimicrobial and antioxidant properties. Though leaves of Cauliflower have a very high waste index, the present study analyzed is an attempt to exploit the potential benefits of this underutilized vegetable leaves there by reducing the food wastage.

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INTRODUCTION

The advancement in science and technology has explored the possibilities of a better medicine to arrive at a conclusion that food can be technologically designed as medicine that can help us stay away from degenerative diseases. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [1] Cauliflower is one such vegetable belonging to the Brassica oleracea species, in the family Brassicaceae that is popular and is considered among the most consumed vegetable in the world. It is an annual plant that reproduces by seed. Typically the head (the white curd) is eaten, while the stalk and surrounding thick, green leaves are discarded. Cauliflower plant is thought to have originated in ancient Asia Minor. The United States, France, Italy, India and China are countries that produce significant quantities of cauliflower. The brassica family is quite cold resistant, making them well adapted to cool season production. The optimum growing temperature ranges between 15 and 22 °C.

Brassicas are known to possess antioxidant activity[2]. The *Brassica* vegetables serves as an excellent source of antioxidants, consumption in large amounts as a regular vegetable worldwide are added advantages. Beneficial health properties are due to the presence of health-promoting compounds such as vitamins, carotenoids, phenols, flavonoids, minerals, and glucosinolates[3]. The content of these compounds in *Brassica* vegetables varies significantly depending on the genotypes of cultivars, the specific plant tissue, fertilization, growing season, and several other environmental factors[4]. Recent reports suggests that cruciferous vegetables act as a good source of natural antioxidants and strong epidemiological evidence shows that presence of such active compounds prevents human body against damage by reactive oxygen species. There is increasing interest given on edible plants especially those that are rich in secondary metabolites commonly called as phytochemicals.

*Corresponding author: **Mary Jenefer Sharmilan P***
Department of Food Service Management and Dietetics Avinashilingam Institute for Home Science and Higher Education for Women University Coimbatore 6410043

Cauliflower is considered as a rich source of dietary fiber and it possesses both antioxidant and anticarcinogenic properties[5].The leaves of cauliflower has a high waste index during post harvesting thus creating a foul odor on decomposition. Disposal of the non-edible portion of cauliflower (cauliflower waste), which contributes to about 45–60% of the total weight of the vegetable, remains a crucial problem[6].Classically, the outer layers and extremities of fruits and vegetables are removed during processing, mainly by peeling and pressing; they comprise essentially stalks, peels, seeds and crashed pulp which still contain large amounts of bioactive molecules and biopolymers, resulting in a considerable nutritional loss[7].

The medicinal value of plants has assumed a more important dimension in the past decades owing largely to the discovery that their extracts contain not only minerals but also a diverse array of secondary metabolites with antioxidant potentials[8]. There is enough research conducted on the floral parts of cauliflower whereas the leaves and stalks of the vegetable are highly ignored and discarded as waste. There is very scanty literature available on the use of vegetable residues, especially cauliflower waste, for food production[9]. Viewing these perspectives on cauliflower vegetable leaves an attempt was made to identify the bioactive compounds present using Gas chromatography Mass spectrometer (GCMS).

MATERIALS AND METHODS

Fresh leaves of cauliflower were purchased in bulk from "Earth Trust" an organic farm in Ooty, Tamil Nadu. The stalks from the leaves were separated and the leaves were then washed in running tap water. The leaves were blanched for 10-15 sec and were spread in a filter paper for two hours at room temperature for the excess water to drain. Later the leaves were dried in hot air oven at 40^0c over night. Hot air oven drying method was applied because oven- dried (40^oC) cauliflower had the highest extraction yield while air-dried (ambient, approx 25^oC) had the lowest[10]. The dried leaves were allowed to cool and was then ground in a mixer and packed in an air tight container.

Sample Preparation

10gm powdered plant material was soaked in 80ml of ethanol overnight and then filtered through Whatmann filter paper No.41 along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with ethanol. The filtrate is then concentrated by flushing nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytocomponents.

GCMS Instrumentation

The chemical compositions of 1ml were investigated through Gas Chromatography Mass Spectrometry/ Electron Ionization (GC-MS/EI) mode. The GC-MS is an Perkin Elmer GC Claurus 500 system which is interfaced to a Mass Spectrometer equipped with a Elite-5 MS fused silica capillary column (30m x 0.25mm x 0.25µm df) composed of 5% Diphenyl and 95% Dimethyl poly siloxane. In Mass Spectrometry, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 µl was employed (split ratio of 10:1). Injector temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C /min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70eV. A scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms were Turbo Mass Version 5.2.0.

Interpretation of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 1, 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The main criteria for selection of suitable ions for an identification of compound should have a high peak area (>0.05%) and should be unique and be well resolved from other ions with the same mass to charge ratio (m/z) in the defined time window. Identification of the compounds indicated by the library search program as being more than 80% and viewed as being likely hits. The name, molecular weight and structure of the components of the test materials were ascertained. The compounds were quantified using peak area normalization.

RESULTS AND DISCUSSION

Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids[11] and alkaloids[12]. It also plays a fundamental role as an analytical technique for quality control and standardization of phyto therapeuticals[13].The GC-MS analysis in the leaves of *Brassica oleracea var.botrytis (*cauliflower*)* revealed the presence of twenty eight compounds (phytochemical constituents) that could contribute the antioxidant and therapeutic benefits of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage were recorded. The phytochemicals identified through GC-MS analysis showed many biological activities which is listed in the below table. The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA and based on data reviewed from articles.

No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %	Compound name	Activity
1.	9.05	α-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	0.79	Lactose	Antiencephalopathic, Antihepatotic, Neoplastic, Sweetener
2.	9.80	Lactose	$C_{12}H_{22}O_{11}$	342	0.37	Lactose	Antiencephalopathic , Antihepatotic, Neoplastic, Sweetener
3.	10.69	Ethanone, 1-(3,4-dimethoxyphenyl)-	$C_{10}H_{12}O_3$	180	1.44	Acetophenone	**Antibacterial,** Fungicide**,** Hypnotic**,** Perfumery, Soporific
4.	10.95	3-Methyl-4-phenyl-1H-pyrrole	$C_{11}H_{11}N$	157	0.37		hypolipidemic,, **antimicrobial,**anti-inflammatory and antitumour activities and inhibit retroviral reverse transcriptases
5.	11.55	Megastigmatrienone	$C_{13}H_{18}O$	190	0.17	Megastigmatrienone	Volatile compound, Aromatic compound
6.	12.39	Phenol, 5-methyl-2-(pyrrol-1-yl)-	$C_{11}H_{11}NO$	173	0.31		hypolipidemic, **antimicrobial,** anti-inflammatory and antitumour activities and inhibit retroviral reverse transcriptases
7.	12.77	1H-Indole-3-acetonitrile	$C_{10}H_8N_2$	156	0.59	Indole-3-acetonitrile	**Antioxidant,** Antiacne**, Antibacterial,** Anticariogenic Antisalmonella, Antiseptic, Antistreptococcic Cancer-Preventive, Carcinogenic, Insectiphile Nematicide, Perfumery;, Fragrance, Pesticide Tumorigenic
8.	13.02	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.39	Mysteric acid	food industry as a flavoring agent important fatty acid
9.	13.25	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	$C_{12}H_{20}O_2$	196	0.54		Major phytochemical responsible for various pharmacological actions like antimicrobial activity
10.	13.45	Pregn-4-ene-3,20-dione, (9β,10α)-	$C_{21}H_{30}O_2$	314	0.67	Progesterone	Menstrual cycle,pregnancy, mammary gland development in females
11.	13.66	2,3'-Dipyridyl	$C_{10}H_8N_2$	156	0.46		2,3'-Bipyridyl
12.	14.00	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.13	Phytol	Phytol, an acyclic terpenoid, is used in manufacturing synthetic vitamins E and K. It is an ingredient of fragrances.
13.	14.55	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.61		
14.	15.29	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	12.51	Linolenic acid	Antiinflammatory, Insectifuge Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective,Insectifuge, Antihistaminic, Antieczemic,Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary
15.	15.61	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.95	Palmitic acid	5-Alpha-Reductase-Inhibitor; Antialopecic,Antiandrogenic Antifibrinolytic, **Antioxidant**, Flavor, Hemolytic
16.	17.68	Phytol	$C_{20}H_{40}O$	296	2.94	Phytol	Phytol is beneficial in regulating blood glucose and can possibly restore the metabolic functions of a type 2 diabetic. There have also been studies regarding the effectiveness of phytol in reducing cholesterol levels in blood ultimately reducing blood pressure levels as well. **Antimicrobial**, Anticancer, Cancer preventive, Diuretic, Antiinflammatory
17.	18.13	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	44.52	Linolenic acid	Antiinflammatory, Insectifuge Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective,Insectifuge, Antihistaminic, Antieczemic,Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary
18.	18.42	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.64	Stearic acid	Saturated fatty acid, antiviral and anti inflammatory properties Flavor, nematicide
19.	18.49	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	2.65		
20.	20.99	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310	0.18	Paullinic acid	A rare omega 7 fatty acid. Reported only in Sapindaceae Family Paullinia elegans (Sapindaceae)
21.	26.19	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	0.46		
22.	27.82	β-Amyrin	$C_{30}H_{50}O$	426	3.28	Beta amyrin	Analgesic , Antiedemic , Antiinflammatory, Antinociceptive Antiulcer, Gastroprotective, Hepatoprotective. Larvicide Mosquitocide

23	31.00	α-Amyrin	$C_{30}H_{50}O$	426	1.03		Analgesic, Antiedemic, Antiinflammatory, Antinociceptive Antitumor, Antiulcer, Cytotoxic, Gastroprotective Hepatoprotective, Insectifuge
24	32.11	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	$C_{22}H_{28}N_2O_3$	368	0.64	Antacid	
25	32.63	Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)-	$C_{25}H_{48}$	348	1.73		
26	32.94	Vitamin E	$C_{29}H_{50}O_2$	430	4.42		
27	35.03	Androstan-17-ol, 2,3-epoxy-, (2α,3α,5α,17β)-	$C_{19}H_{30}O_2$	290	0.79		
28	35.71	Stigmasterol	$C_{29}H_{48}O$	412	0.07	Stigmasterol, Phtosterol	An essential component of cell membrane to play a role in the inhibition of tumour growth and control of cholesterol in the blood.

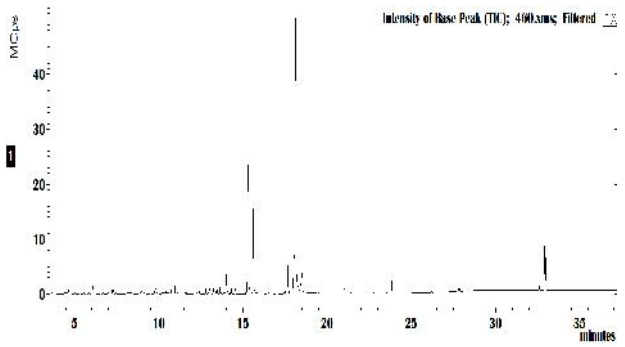

Figure 1 Graphical representation of peak intensity of compounds

DISCUSSION

The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS) [14]. The GC-MS analysis revealed the potential bioactivities of ethanolic extracted Brassica *oleracea var. botrytis* leaves (cauliflower). The results showed presence of very strong health promoting fatty acids antioxidants and antimicrobial activities which could be successfully applied in food industry and pharmaceutical industries. Among the twenty eight compounds identified 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) (linolenic acid), an essential fatty acid commonly called as omega 3 fatty acid showing a peak area of 44.5 % (R/T 18.13) possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. Following this comes n-Hexadecanoic acid - palmitic acid (R/T 12.95) an essential fatty acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Ethanone, 1-(3,4-dimethoxyphenyl)-(R/T10.69), 3-Methyl-4-phenyl-1H-pyrrole (R/T10.95), Phenol, 5-methyl-2-(pyrrol-1-yl)-(R/T12.39), 1H-Indole-3-acetonitrile(R/T12.77), phytol (R/T17.68) are notable compounds possessing antimicrobial activities. 1H-Indole-3-acetonitrile, n-Hexadecanoic acid is notable compounds identified possessing antioxidant activities. The results revealed the presence of fatty acids, antioxidants and antimicrobial activities. The study also revealed the potential bioactivities of cis-13-Eicosenoic acid (Paullinic acid), a rare omega 7 fatty acid. Reported only in Sapindaceae Family Paullinia elegans (Sapindaceae)[15]. The leaf powder also confirmed the presence of other promising compounds possessing anti cancer and anti tumour activities.

A large number of Indian villager's uses traditionally acquired knowledge for treatment of various ailments by using locally available plants and plant products[16]. Supporting the above reference this article strongly supports that cauliflower is a common vegetable that is easy to avail all time and but its leaves are usually discarded showing a very high waste index and hence these leaves can be treated as a traditional medicine to fight against degenerative diseases and can also act as a precursor in maintaining good health. The leaves can also be technically applied in food processing sector due to its antioxidant and antimicrobial properties.

CONCLUSION

There is a great interest in plant and plant derived phytochemical as food source because of its divergent nutritional, functional, antioxidant and other therapeutic properties[17]. GC-MS analysis showed the existence of various compounds with different chemical structure. Today there is increased concern on the application of natural antioxidants in place of synthetic antioxidants in food and pharma industry. In response to these concerns this research is the first step towards understanding and exploiting the nature of active principles present in cauliflower leaves thereby creating awareness on reducing the waste index of this underutilized leaves and ventures into a new direction that these leaves can readily be used in pharma sectors and in food industries. Hence the present study suggests that cauliflower leaves can be strongly recommended in developing potential health and food products for safe consumption due to its promising bioactive compounds.

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