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Research Article

DETERMINATION OF PHYTOCOMPONENTS IN ETHANOL EXTRACT OF *BRASSICA OLERACEA* - USING GAS CHROMATOGRAPHY–MASS SPECTROSCOPY TECHNIQUE

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ABSTRACT

Objectives: Bioactive components determined by plants are known to have a broad application in the medical field. The focus of this study is to recognize the phytochemicals in the ethanol extract of *Brassica oleracea* by gas chromatography–mass spectroscopy (GC–MS).

Methods: *B. oleracea* was collected, dried, and powdered well. The extraction was done with the solvent ethanol. The extract was exposed to column of GC-MS-QP 2010 (SHIMADZU) column Db 30.0 (0.25 µm in diameter, 0.25 µm thick).

Results: GC–MS result provides the chromatogram with different peaks obtained at a different retention time shows the presence of various biocompounds. Some of the identified bioactive compounds are n-hexadecanoic acid (12.99%), phytol (2.40%), Vitamin E (3.38%), tetratetracontane (2.15%), stigmasterol (2.03%), and isophytol.

Conclusion: The GC–MS study of the ethanol extract of *B. oleracea* reveals the existence of many potential compounds that can be utilized in the pharmaceutical industry, including the use of anti-inflammatory, antiarthritic, anticoronary, and antidiabetic agents.

Keywords: *Brassica oleracea*, Gas chromatography–mass spectroscopy, Tetratetracontane, Stigmasterol, Phytocompounds, antioxidant.

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INTRODUCTION

Plant sources play a substantial role in the drug development industry. The developing countries are recommended to use ancient way of herbal medicines by the World Health Organization to treat the various chronic illnesses [1]. In India, accustomed systems of medical treatment such as Unani, Ayurveda, and Siddha are established on the herbal drugs [2]. The active biocomponents of the therapeutically valued plants are expansively used in treating the mild or chronic disorders [3]. In plants, every portion of the bark, leaves, flowers, roots, seeds, and fruit is acting as the sources for the biologically active biocompounds [4].

Nowadays, researchers have diversified their scrutiny toward the herbal side for the finding of new drugs because of the less toxicity and cost-effective. Many of the compounds demonstrate a foremost role as metallic enzymes and as enzyme cofactors [5,6]. *Brassica oleracea* is a pharmaceutical valued plant which comes under the botanical family Cruciferae. Broccoli features are look-alike cauliflower, and it is highly valuable and desirable food among the Italians [7]. Broccoli is the remarkable healthy diet item which is well off in protein, beta-carotene, Vitamin C, Vitamin B6, potassium, and calcium, and they are high in fiber [8].

Broccoli is enriched with the plenty of metabolites such as flavonoids, glucosinolates, glucoraphanin, glucoiberin, and sulforaphane. These constituents of broccoli show that Broccoli exhibits high therapeutic properties and are used in curing various chronic diseases. Broccoli is found to play a significant part in the treatment of arthritis. Broccoli is also tangled in the maintaining the insulin level and plays an extensive role in treating the kidney diseases [9].

Gas chromatography–mass spectroscopy (GC–MS) is the finest method to scrutinize the biologically active constituents, namely alcohols, long-chain hydrocarbons, branched chain hydrocarbons, esters, etc. GC is the preferable tool on account of its sensitivity, effective in separating the constituents from the mixture and also used for the qualitative and quantitative study of the mixture. By GC–MS, we can also record a mass spectrum of each component [10]. Hence, this paper is focused on the detection of biocompounds present in broccoli by GC–MS analysis and to assess its pharmacological properties.

METHODS

Collection of the plant material

The edible parts of *B. oleracea* were collected from the local marketplace and it was washed well. The edible parts were cut into a small piece and shade dried for about 7 days. The dried parts were powdered well, which further got into extraction process.

Authentication of the plant material

The plant material *B. oleracea* was collected and authenticated in ICAR by Dr. N. Kaliaperumal M.Sc., Ph.D., Scientist-in-charge, CMFRI.

Preparation of the plant extract

The sample powder was extracted using 99.8% ethanol solvent. The sample was submerged in ethanol and it was incubated for 72 h inside an incubator. After the incubation period, it was filtered using a muslin cloth, and the filtrate was kept open in the closed space for the ethanol to evaporate completely. The remains of the filtrate were the crude ethanol extract of broccoli, which was maintained at 4°C for analytical purposes.

GC–MS

GC–MS technique was performed to analyze the phytocomponents exist in the ethanol extract of Broccoli. This technical process was done in the SMS Laboratory, Thiruvallur, Tamil Nadu. Chromatographic separation was performed using a column of GC-MS-QP 2010 (SHIMADZU) column Db 30.0 (0.25 µm in diameter, 0.25 µm thick). The oven temperature is raised to 10°C/min to 200°C and then programmed to 5°C/min to 280°C and 70°C (isothermal 5 min) ending to 35° isothermal. Obtained at 70

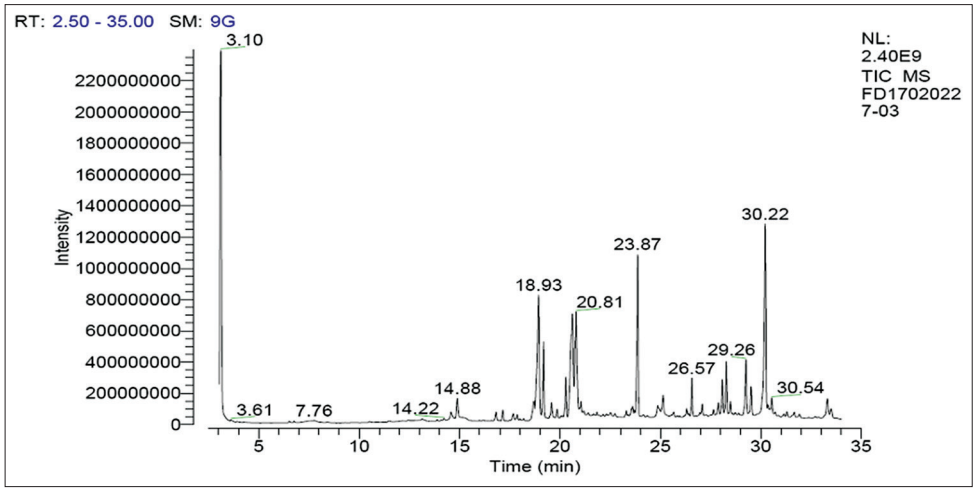


Fig. 1: Chromatogram of ethanol extract of *Brassica oleracea*

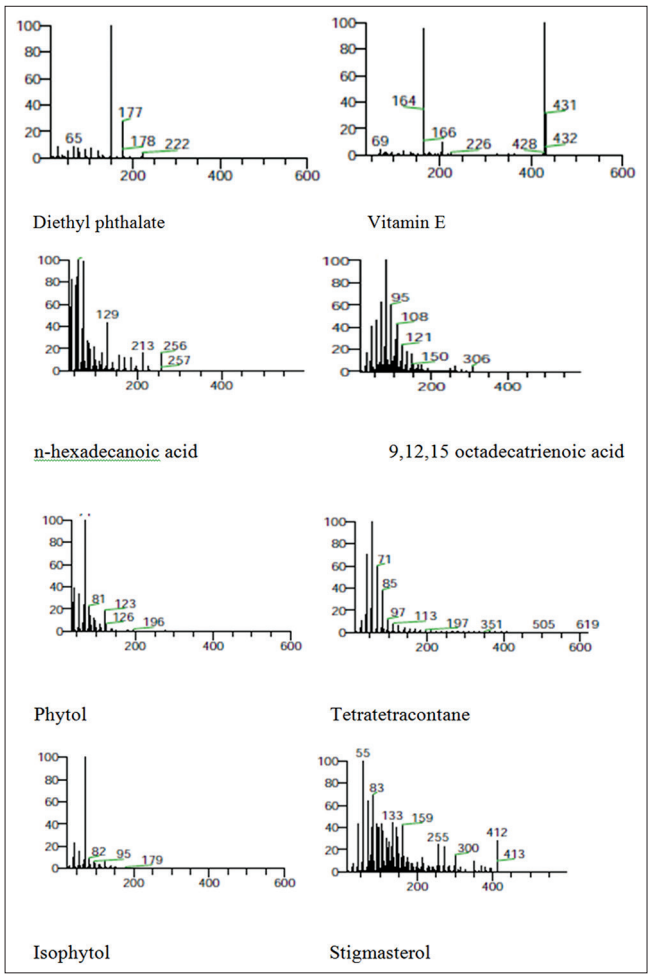


Fig. 2: Mass spectrum of identified compounds

eV. The range of the obscure segment was contrasted with the range of standard components put away in the NIST library. Helium acted as a carrier with a gas pressure of 99.999% and a flow rate of 1.0 ml/min and electronic pressure control. The sample was added automatically.

Identification of compounds

The elucidation of GC–MS mass range was made utilizing the National Institute of Standards and Technology (NIST) database, which has in excess of 62,000 models. The range of the obscure segment was contrasted with the range of standard components put away in the NIST library. The name, molecular weight, and structure of the components of the test materials have been established [11].

RESULTS AND DISCUSSION

GC–MS is the ideal method used for the resolution of volatile compounds. This technique has been the frequently used methods for examining plant samples. In general, GC–MS analysis provides the idea about the chemical structure, molecular formula, and idea about the functional group present in the compound [12].

The GC–MS results of an ethanol extract of *B. oleracea* reveal the appearance of a number of bioactive compounds. The chromatogram of ethanol extracted from *B. oleracea* is shown in Fig. 1. The most common compounds in the ethanol extract of *B. oleracea* are diethyl phthalate, 9, 12, 15 octadecatrienoic acid, pentadecanoic acid, Vitamin E, stigmasterol, phytol, isophytol, and tetratetracontane. The phytochemical composition of the ethanol extract of *B. oleracea* with compound name, molecular formula, molecular structure, retention time, and peak area was shown in Table 1, and its biological activity with its structure was given in Table 2.

Diethyl phthalate was detected at the retention time of 14.88 with its peak area of 78% and the compound was revealed to possess antifungal and antimicrobial activity. Olawale *et al.* stated the existence of this compound in *Pycnanthus angolensis* and proved its antibacterial activity against *Escherichia coli* [13].

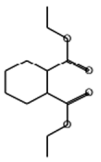
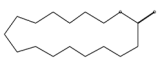
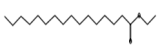
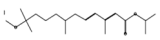
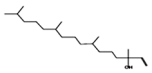
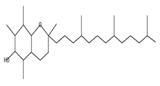
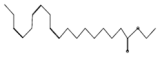
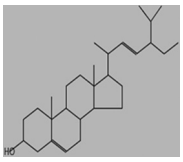
Jagadeeswari *et al*. reported the documentation of the compound n-hexadecanoic acid in *Aristolochia krysagathra.* n-hexadecanoic acid has pharmacological activities such as antimicrobial, antioxidant, hypocholesterolemic, antiarthritic, and anti-inflammatory [14]. Phytol was identified at the retention time of 20.29 with the peak area of 2.40%. de Freitas *et al*., 2013, demonstrated the antinociceptive activity associated with phytol antioxidant activity by *in vitro* methods [15]. The mass spectrum of different compounds is shown in Fig. 2.

It is well known that isophytol has an antioxidant property and has been identified at a retention time of 20.29 with a peak area of 2.40% [16]. Vitamin E is a significant vitamin for the human immune system that is naturally present in some food products and can also be obtained in the diet of a supplement capsule. It belongs to a class of compounds that include tocopherol and tocotrienols. Vitamin E includes an antioxidant, antidiabetic, anti-inflammatory, and antiaging process [17]. 9,12,15 octadecatrienoic acid was identified at a retention time of 20.81 with a peak area of 11.08%. It includes anti-inflammatory,

Table 1: The phytochemical composition of ethanolic extract of *Brassica oleracea* with the compound name, its molecular formula, molecular structure, retention time, and peak area

S. No	RT	Compound name	Molecular formula	Molecular weight	Peak area%
1	14.88	Diethyl phthalate	$C_{12}H_{14}O_4$	222	1.78
2	18.70	Palmitoleic acid	$C_{16}H_{30}O_2$	254	2.09
3	18.70	Oxacycloheptadecan-2-one	$C_{16}H_{30}O_2$	254	2.09
4	18.70	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	2.09
5	18.93	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.99
6	18.70	Erucic acid	$C_{22}H_{42}O_2$	338	2.09
7	19.18	Hexadecanoic acid, Ethyl ester	$C_{18}H_{36}O_2$	284	4.51
8	19,18	Pentadecanoic acid, Ethyl ester	$C_{17}H_{34}O_2$	270	4.51
9	19.57	Heptadecanoic acid	$C_{19}H_{38}O_2$	298	4.51
10	20.29	Phytol	$C_{20}H_{40}O$	296	2.40
11	20.29	Isophytol	$C_{20}H_{40}O$	296	2.40
12	20.81	9,12,15 Octadecatrienoic acid, ethyl ester	$C_{20}H_{34}O_2$	306	11.08
13	23.87	bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	10.67
14	25.13	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	288	1.81
15	26.57.	Tetratetracontane	$C_{44}H_{90}$	618	2.15
16	28.28	Vitamin E	$C_{29}H_{50}O_2$	430	3.38
17	29.26	Campesterol	$C_{28}H_{48}O$	400	4.10
18	29.26	Cholesterol, 7-Oxo-	$C_{27}H_{44}O_2$	400	4.10
19	29.51	Stigmasterol	$C_{29}H_{48}O$	412	2.09
20	33.32	Methoprene	$C_{19}H_{34}O_3$	310	2.03
21	33.32	Dodecane, 1,12dibromo	$C_{12}H_{24}Br_2$	326	2.03

Table 2: Bioactivity of phytocomponents with its structure identified in the ethanol extract of *Brassica oleracea* by GC–MS

S. No	Name of the compound	Structure	Biological activity
1.	Diethyl phthalate		Antimicrobial activity, antifungal activity
2.	Palmitoleic acid		Not intended for therapeutic purposes
3.	Oxacycloheptadecan-2-one		Not intended for therapeutic uses
4.	n-Hexadecanoic acid		Antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, and anti-inflammatory activities
5.	Methoprene		Not intended for therapeutic uses
6.	Phytol		Antimicrobial, anticancer, diuretic, anti-inflammatory
7.	Isophytol		Antioxidant activity
8.	Vitamin E		Antiageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer
9.	9,12,15 Octadecatrienoic acid, Ethyl ester (Z, Z, Z)		Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antibacterial, antiarthritic, and anticoronary activities
10.	Octadecane, 1-chloro-		Not intended for therapeutic purposes
11.	Tetratetracontane		Antioxidant and cytoprotective activities
12.	Stigmasterol		Antiosteoarthritic, antihypercholestrolemic, antitumor, hypoglycemic, antimutagenic, antioxidant, anti-inflammatory activities

GC–MS: Gas chromatography–mass spectroscopy

anticancer and hypocholesterolemic, hepatoprotective, antibacterial, antiarthritic, and anticoronary activities. Sermakkani *et al*. described the occurrence of this compound in the *Cassia italica* leaf and reported its pharmacological activity [18].

Tetratetracontane was identified at a retention time of 25.13 with a peak area of 2.15%. Mallick *et al*. identified this compound by the high-performance thin-layer chromatography technique and represents the antioxidant and cytoprotective properties [19]. Stigmasterol is well known by its other name called Wulzen anti-stiffness factor is a type of unsaturated plant sterols. Stigmasterol was reported by Gabay. O for its anti-osteoarthritic activity. Stigmasterol includes antimutagen antioxidant and anti-inflammatory and also plays a role in regulating cholesterol biosynthesis by inhibiting Δ24 reductase [20]. Nowadays, developing the drugs with improved tolerance and/or specificity is an imperative goal for the drug industry, which can be achieved from herbal plants [21].

CONCLUSION

The GC–MS result of the ethanol extract of *B. oleracea* exaggerates the manifestation of more than 20 phytocompounds. It explores the persistence of numerous bioactive constituents which have the ability to act as a prospective substance of drugs in the pharmaceutical industry. This proves that *B. oleracea* can be utilized to treat the various ailments and is of pharmaceutical significance. However, further research is required to elute the novel bioactive compounds.

AUTHOR'S CONTRIBUTION

No author's contribution.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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