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

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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, acusttomed 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 biocomponents [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, glucobrassicin, 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 bio components 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 IGR 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 phyto components exist in GC–MS 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
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 extract of *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, anti-diabetic, 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              | C₁₂H₁₄O₄          | 222              | 1.78       |
| 2     | 18.70| Palmitoleic acid               | C₁₆H₃₀O₂          | 254              | 2.09       |
| 3     | 18.70| Oxacyloheptadecan-2-one        | C₁₆H₃₀O₂          | 254              | 2.09       |
| 4     | 18.93| n-Hexadecanoic acid            | C₁₆H₃₀O₂          | 256              | 12.99      |
| 5     | 18.70| Erucic acid                    | C₂₂H₴₂O₂          | 338              | 2.09       |
| 6     | 19.18| Hexadecanoic acid, Ethyl ester | C₁₆H₃₀O₂          | 284              | 4.51       |
| 7     | 19.18| Pentadecanoic acid, Ethyl ester| C₁₇H₃₄O₂          | 270              | 4.51       |
| 8     | 19.57| Heptadecanoic acid             | C₁₇H₃₄O₂          | 298              | 4.51       |
| 9     | 20.29| Phytol                         | C₂₀H₄₀O          | 296              | 2.40       |
| 10    | 20.29| Isophytol                      | C₂₀H₄₀O          | 296              | 2.40       |
| 11    | 20.81| 9,12,15 Octadecatrienoic acid, | C₂₀H₃₄O₂          | 306              | 11.08      |
| 12    | 23.87| bis (2-ethylhexyl) phthalate   | C₂₀H₄₄O₂          | 390              | 10.67      |
| 13    | 25.13| Octadecane, 1-chloro-          | C₁₈H₃₇Cl          | 288              | 1.81       |
| 14    | 26.57| Tetratetracontane              | C₄₄H₹₀O          | 618              | 2.15       |
| 15    | 28.28| Vitamin E                      | C₂₀H₄₀O          | 430              | 3.38       |
| 16    | 29.26| Campesterol                    | C₂₈H₄₈O₂          | 400              | 4.10       |
| 17    | 29.26| Cholesterol, 7-Oxo-            | C₂₇H₄₄O₂          | 400              | 4.10       |
| 18    | 29.51| Stigmasterol                   | C₂₉H₄₈O₂          | 412              | 2.09       |
| 19    | 33.32| Methoprene                     | C₁₉H₃₄O₂          | 310              | 2.03       |
| 20    | 33.32| Dodecane, 1,12dibromo          | C₁₂H₂₄Br₂          | 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    | ![Image](#) | Antimicrobial activity, antifungal activity                                          |
| 2     | Palmitoleic acid     | ![Image](#) | Not intended for therapeutic purposes                                               |
| 3     | Oxacyloheptadecan-2-one | ![Image](#) | Not intended for therapeutic uses                                                   |
| 4     | n-Hexadecanoic acid  | ![Image](#) | Antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, and anti-inflammatory activities |
| 5     | Methoprene           | ![Image](#) | Not intended for therapeutic uses                                                   |
| 6     | Phytol               | ![Image](#) | Antimicrobial, anticancer, diuretic, anti-inflammatory                                |
| 7     | Isophytol            | ![Image](#) | Antioxidant activity                                                               |
| 8     | Vitamin E            | ![Image](#) | Antiageing, analgesic, anti-diabetic, anti-inflammatory, anti-arthritic, antitumor, anticancer |
| 9     | 9,12,15 Octadecatrienoic acid, Ethyl ester (Z, Z) | ![Image](#) | Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antibacterial, antiarthritic, and anticoagulant activities |
| 10    | Octadecane, 1-chloro- | ![Image](#) | Not intended for therapeutic purposes                                               |
| 11    | Tetratetracontane    | ![Image](#) | Antioxidant and cytoprotective activities                                            |
| 12    | Stigmasterol         | ![Image](#) | Antiosteoarthritic, antihypercholesterolemic, antitumor, hypoglycemic, antimutagenic, antioxidant, anti-inflammatory activities |

GC–MS: Gas chromatography–mass spectroscopy
Activation of NF-E2-related factor-2 reverses antinociceptive activity 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.

**REFERENCES**

1. Vasanthi HR, ShriShriMal N, Das DK. Phytochemicals from plants to combat cardiovascular disease. Curr Med Chem 2012;19:2242-51.
2. Ravishankar B, Shukla VJ. Indian systems of medicine: A brief profile. Afr J Tradit Complement Altern Med 2007;4:319-37.
3. Varadharaj V, Muniyappan J. Phytochemical and phytotherapeutic properties of Celosia species—a review. Int J Pharm Pharm Res 2017;9:820-5.
4. Gordon DM. Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination.

**Microbiology** 2001;147:1079-85.
5. Vanitha J. Betaine supplementation for various clinical disorders. Asian J Pharm Clin Res 2017;10:27-31.
6. Vanitha V, Jayalakshmi M, Annadha P, Pushpaharathni N. Assesment of minerals from shell waste of Peneus indicus. Int J Res Pharm Sci 2017;8:194-7.
7. Webster M. Broccoli. Merriam-Webster’s Collegiate Dictionary. 11th ed. America: ???; 2014:156.
8. Wallenbring LW. Inhibition of carcinogenesis by non-nutrientconstituents of the diet. In: Food and Cancer Prevention. Chemicaland Biological Aspects. London: The Royal Society of Chemistry; 1993. p. 12-24.
9. Xue M, Qian Q, Adaikalakoteswari A, Rabbani N, Babaei-Jadidi R, Thornalley PJ, et al. Activation of NF-κB-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. Diabetes 2008;57:2809-17.
10. Krishnakumari ST, Muthukumarasamy S, Mohan VR. GC-MS analysis of ethanol extract of Sarcostemma secamone(L) Bennet (Asclepiadaceae). Sci Res Rep 2012;2:187-91.
11. Mathi P, Nikhil K, Das S, Roy P, Bokka VR, Botlagunta M. Evaluation of in vitro anticancer activity and GC-Ms analysis from leaf Sophora interrupta bedd. Int J Pharm Pharm Sci 2015;7:303-8.
12. Khumukcham N, Ajungle T, Singh CB. GCMS based metabolic profiling of essential oil of Citrus macroptera montnae. Leaves and peel, assessment of in vitro antioxidant and anti-inflammatory activity. Int J Pharm Pharm Sci 2017;9:107-14.
13. Oladimeji OH, Attih EE, Onu NO. Ethyl linalool and diethyl phthalate from pycnanthus angolensis (Welw.) varb. Eur Chem Bull 2017;6:76-8.
14. Jegadeeswari P, Nishanthini A, Muthukumarasamy S, Mohan VR. GC-MS Analysis of bioactive components of Aristolochia krysantha (Aristolochiaceae). J Curr Chem Pharm Sci 2012;2:226-32.
15. de Freitas RM, de Menezes Patricio Santos CC, Salvadori MS, Mota VG, Costa LM, de Almeida AA, et al. Antinociceptive and antioxidant activities of phytol in vivo and in vitro models. Neurosci 2013;2013:Article ID 949452.
16. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, et al. Cyclooxygenase in biology and disease. In: Food and Cancer Prevention. Chemical and Biological Aspects. London: The Royal Society of Chemistry; 1993. p. 12-24.
17. Dr. Duke’s Phytochemical and Ethnobotanical Databases. Available from: http://www.ars-grin.gov/duke/. [Last accessed on 2012 Aug 10].
18. Sermakkani M, Thangapandian V. GC-MS analysis of Cassia italica leaf methanol extract. Asian J Pharm Clin Res 2012;5:90-4.
19. Mallick SS, DigheVV. Detection and estimation of alpha-amyrin, beta-sitosterol, lupeol and n-tricontane in two medicinal plants by high performance thin layer chromatography. Adv Chem 2014;2014:Article ID 143948.
20. Kaar N, Chaudhary J, Jain A, Kishore L. Stigmasterol: A comprehensive review. Int J Pharm Sci Res 2011;2:2259-65.
21. Casucci I, Provenzani A, Poldori P. Evaluation of treatment of invasive fungal infections. J Pharmaco Pharmother 2014;5:47-52.