ORIGINAL ARTICLE

Preliminary Study of Antioxidant Potential and Gas Chromatography-mass Spectroscopy (GC-MS) Analysis of *Brassica oleracea* Florets

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Abstract

Introduction: *Brassica oleracea var. Italica* ordinarily known as "broccoli" is a cruciferous green leafy vegetable and has been reported to have antioxidant, anti-inflammatory, antibacterial, anticancer, antiatherosclerotic, antimucolytic, antinitrosaminic, anti-nyctalopic, antiproliferant, and hypocholesterolemic potential. To determine the presence of bioactives, preliminary phytochemical screening of methanol extract was done and its antioxidant potential was evaluated by *in vitro* radical scavenging assays. Phytoconstituents present in the extract were identified by gas chromatography-mass spectroscopy (GC-MS) analysis. **Materials and Methods:** In the preliminary phytochemical screening, various chemical tests were performed. Evaluation of the *in vitro* antioxidant efficacy of the extract was done by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging assay and ferrous ion-chelating ability assay. Various phytoconstituents were identified on the basis of retention indices and mass spectra fragmentation pattern obtained through GC-MS studies. **Results:** Phytochemical screening of the floret extract revealed the presence of terpenoids, alkaloids, tannins, steroids, and saponins. Plant has significant scavenging effect on DPPH (half maximal inhibitory concentration [IC_{50}] = 0.070 ± 0.67 mg/ml) which is comparable to that of ascorbic acid (IC_{50} = 0.045 ± 0.61 mg/ml) and butylated hydroxytoluene (BHT) (IC_{50} = 0.118 ± 0.53 mg/ml). The highest chelating activity of methanol extract and ethylenediaminetetraacetic acid is found to be 83.69% and 98.91%, respectively. GC-MS analysis report confirmed the presence of ascorbic acid 2,6-dihexadecanoate, phytosterols, linoleic acid, palmitic acid, and oleic acid as the major constituents. Terpenoids, namely squalene and geraneol, were found in traces. **Discussion and Conclusion:** From the study, it has been concluded that the *B. oleracea* is a potential source of plant-based therapeutics and a natural source of antioxidants. It has been found that the extract contains a significant amount of phytochemicals with antioxidants which could act as scavengers of free radicals. From this study, we got a sound base for further investigation of *B. oleracea* for its pharmaceutical application.

Key words: 1,1-Diphenyl-2-picrylhydrazyl, antioxidant, fluorescence recovery after photobleaching, gas chromatography-mass spectroscopy, phytochemical screening

INTRODUCTION

Plant plays a vital role for the healthfulness of the human being and is the major source of medicinally important compounds. Since the ancient time, people all around the world are using indigenous flora for medicinal purpose. *Brassica oleracea* or broccoli is one of the prominent edible herbs and has been categorized as functional food as it contains normal dietary constituents and has plenty of therapeutically active components as well which are helpful in delaying or preventing a large number of ailments.[1] *B. oleracea* has health-promoting properties such as antioxidant, anticarcinogenic, anti-atherosclerotic, antibacterial, anti-maculitic, anti-nitrosaminic, anti-nyctalopic, antiproliferant, antioxidant, anti-radicular, anti-retinitic, antitumor, antiviral,

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detoxicant, estrogenic, glucuronidase inhibitor, goitrogenic, hypocholesterolemic, prooxidant, quinone-reductase-inducer, and anticonvulsant.[2,3] It is mainly composed of polyphenols, glucosinolates, sulforaphane, and selenium.[4]

Broccoli is a low-calorie vegetable; it provides just 34 calories per 100 g.[5,6] Broccoli have plethora of medicinally important compounds such as alkaloids, tannins, flavonoids, and phenols which have antioxidants, antibacterial, anti-inflammatory, anticancer, and hepatoprotective potential. Antioxidants are the active compounds which have the capability of neutralizing the free radicals present inside the human body. Free radicals are the reactive species produced in metabolic process and by air pollution, smoking, and radiation in human body. These free radicals react with various cell organelles may cause cell death or chronic diseases such as cardiovascular dysfunctions, neurodegeneration, aging, and weakening of immune system.[7] Chemical compounds present in the plant as secondary metabolites and have various structural arrangements. For the therapeutic knowledge of the reported compounds and also for the exploration of new compounds, analysis of chemical components of herbals must be done. Preliminary phytochemical screening is quite useful in the detection of the bioactive principles. To further carry out drug discovery and spectroscopic studies, mass spectrometry (MS), coupled with chromatographic separations technique like gas chromatography-MS (GC-MS), is a very good technique.[8] On the basis of above facts, the current study was planned to perform phytochemical screening and evaluate antioxidant potential of using various assays such as 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and fluorescence recovery after photobleaching (FRAP). Besides, identification of phytoconstituents was done by GC-MS analysis taking the retention indices (RIs) and mass fragmentation pattern of each compound into consideration. RIs or Kováts index of an analyte is its relative time position between the nearest *n*-alkanes which elute immediately before and after a target analyte. In the GC-MS, retention time (Rt) is the prime variable considered, but it is a function of experimental conditions and has low reproducibility. Moreover, for the identification of branched alkyl substituents, mass spectral differences are not significant. Combination of retention data with MS provides accurate identification of the compounds. Comparing the known RIs from a retention-data library with measured values is the standard approach to identification.[9]

MATERIALS AND METHODS

Collection and identification of plant material

Florets of *B. oleracea* were collected from open fields, Sikanderpur, Sirsa, Haryana, in December 2016 and identified by Dr. Anjula Pandey, Principal Scientist, ICAR- National Bureau of Plant Genetic Resources, Pusa Campus New Delhi, vide reference no. NHCP/NBPGR/2017-23. The plant was identified as *B. oleracea* var. *italica* Plenck, (Brassicaceae). A voucher specimen is retained in the Department of Pharmaceutical Sciences, Guru Jambeshwar University of Sciences and Technology, Hisar, for future reference. Florets were used to carry out the experimental work procedures pertaining to phytochemical and *in vitro* antioxidant evaluations.

Preparation of extracts

500 g of air-dried crude powder of florets of *B. oleracea* was defatted first with petroleum ether (60°C–80°C) for 7 days by cold maceration. The defatted drug was then extracted with methanol (95%) as solvent by continuous hot percolation in Soxhlet apparatus for 72 h. Solvent was removed using rotary vacuum evaporator and a semi-solid mass was obtained which is stored in desiccators for further use.

Preliminary phytochemical screening

Methanol extract of *B. oleracea* was subjected to phytochemical tests for terpenoids, alkaloids, tannins, steroids, and saponins.[10-14]

In vitro antioxidant assays

DPPH radical scavenging assay

Antioxidant potential of extract was estimated by determining its DPPH radical scavenging capability by adopting the method of Roy *et al.*[15] with minor modifications. The dried methanol extract was diluted from 0.02 mg/ml to 0.12 mg/ml. 1 ml of 0.135 mM DPPH solution (in methanol) was added to 1.0 ml of extract (in methanol). This mixture was vortex mixed and incubated in the dark for 30 m at room temperature. Scavenging of DPPH radical by the extract solution converts the DPPH solution from violet to orange, yellow or pale yellow color. Absorbance of the mixture was measured at 517 nm. Ascorbic acid and butylated hydroxyl toluene (BHT) were taken as standards. Scavenging of DPPH radicals by the extract was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Abs of control} - \text{Abs of sample})}{\text{Abs of control}} * 100 \quad (1)$$

Where Abs of control is the absorbance of DPPH only.

Abs of sample is the absorbance of the DPPH radical + sample extract/standard.

Metal ion chelating ability assay

The chelating ability of ferrous ion by the plant extract was estimated by adopting the method followed by Rajauria *et al.*[16] with some modifications. In this assay,

blue-colored ferrous ion-ferrozine complex is formed which has a maximum absorbance at 562 nm. In the assay procedure, 100 µl of different concentrations of extract samples/standard was mixed with 100 µl of deionized water. 25 µl of ferrous chloride (0.5 mm) was added to initiate the reaction. Then, the mixture was shaken vigorously and incubated at ambient temperature for 10 min. Absorbance was recorded at 562 nm. Ethylenediaminetetraacetic acid (EDTA) was used as a standard. The percentage of inhibition of ferrozine– Fe2+ complex formation was calculated using equation (1).

GC-MS analysis

For GC-MS analysis, sample was prepared by dissolving the dried extract in methanol and then filtered with filter paper (Whatman No. 42) to obtain a clear solution. GC-MS was carried out on a GC-MS-QP2010 Plus (Shimadzu, Kyoto, Japan) system with attached auto-injector (AOC-20i) and with headspace sampler (AOC-20s). Column used for separation was a Rtx 5 MS capillary column (Restek Company, Bellefonte, USA: Crossbond 5% diphenyl/95% dimethyl polysiloxane) having dimensions 30 m (length) × 0.25 mm (diameter) × 0.25 µm (film thickness). Detector was mass selective detector with an ion source of temperature 230°C, interface temperature of 260°C, a solvent cut time of 2.50 min, threshold of 1000 eV, and mass range of 40–650 m/z. The split mode was used at a ratio of 10:1. Temperature of the injector was initialized to 250°C, having a split injection mode. Temperature was programmed from 100°C (3 min) and then further increased to 280°C at a ramp rate of 10°C/min (19 min hold). Carrier gas used was helium (>99.999%) with a linear flow velocity of 40.9 cm/s. The debit of gas (helium) vector was fixed to 16.3 mL/min, with a total flow of 1. 21 mL/min. 1 µL of sample was injected. The components were identified by comparison of their RIs relative to homologous alkane series (purchased from Sigma, St. Louis, USA) and by comparison of their mass spectral fragmentation patterns with those data provided in WILEY8.LIB, NIST08.LIB, NIST08s.LIB, and NIST.LIB. Identification was assumed when a good match of mass spectrum and RI was achieved.

RESULTS

Preliminary phytochemical screening

Chemical tests have shown positive results for terpenoids, alkaloids, tannins, steroids, and saponins.

In vitro antioxidant potential

DPPH radical scavenging assay

The DPPH scavenging assay has been widely used to asses antioxidant properties. The concentration-response curve of DPPH radical scavenging activity of the plant extracts, BHT, and ascorbic acid is shown in Figure 1. The flower extract has shown more scavenging than BHT; however, ascorbic acid is more active than the extract. Ascorbic acid has highest inhibition (93%), as compared to that of extract (73%) and BHT (55%). The half maximal inhibitory concentration (IC_{50}) values (the IC_{50} denoted the concentration of sample required to scavenge 50% of DPPH free radicals.) obtained for extract, ascorbic acid, and BHT were 0.070 ± 0.67 mg/ml, 00.045 ± 0.61 mg/ml, and 0.118 ± 0.53 mg/ml, respectively [Table 1].

Metal ion chelating ability assay

Metal ion chelating efficacy of the plant extract is comparable with that of EDTA (used as standard). Plant extract has shown 83.69% inhibition, and EDTA has shown 98.91 % inhibition at 0.12 mg/ml concentration. IC_{50} of extract is 0.061.09 ± 0.53 and that of EDTA is 0.056.69 ± 0.32 [Table 1 and Figure 2].

GC-MS analysis

GC-MS analysis of *B. oleracea* extract resulted in the identification of 25 compounds [Table 2]. Figure 3 shows GC-MS chromatogram of floret extract. Major phytoconstituent identified was hexadecanoic acid also called as ascorbic acid 2,6 dihexa decanoate. Many fatty acids along with their esters have also been identified such as heptadecanoic acid, octadecadienoic acid, tetradecanoic

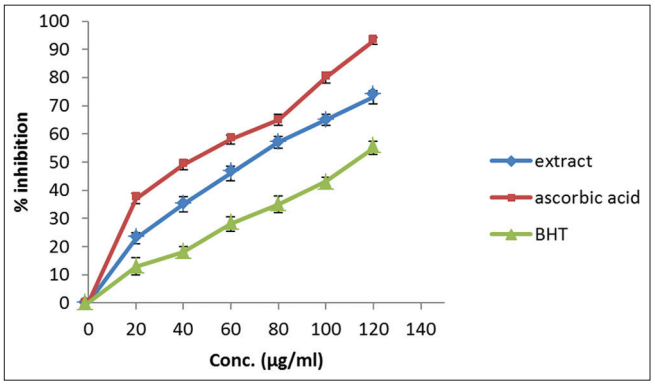

Figure 1: 1,1-Diphenyl-2-picrylhydrazyl scavenging activity of the plant extract and standards

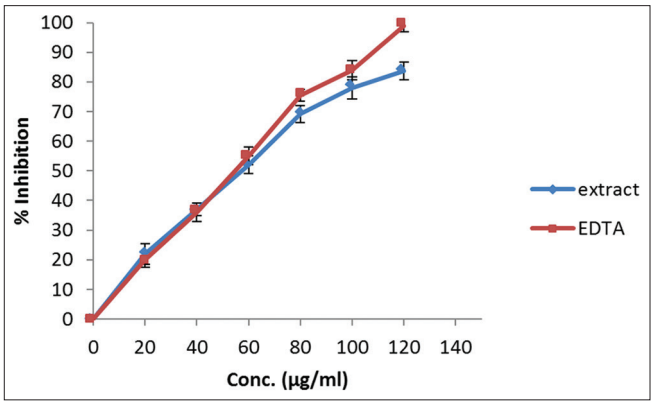

Figure 2: Metal chelating activity of the plant extract and standards

Table 1: DPPH free radical scavenging and ferrous ion-chelating capacity of extract, ascorbic acid, BHT, and EDTA

Samples	Extract	Ascorbic acid	BHT	EDTA
DPPH (IC_{50} in mg/ml)	0.070±0.67	0.045±0.61	0.118±0.53	-
FRAP (IC_{50} in mg/ml)	0.061.09±0.53	-	-	0.056.69±0.32

BHT: Butylated hydroxytoluene, EDTA: Ethylenediaminetetraacetic acid, DPPH: 1,1-Diphenyl-2-picrylhydrazyl, FRAP: Fluorescence recovery after photobleaching, IC_{50}: Half maximal inhibitory concentration

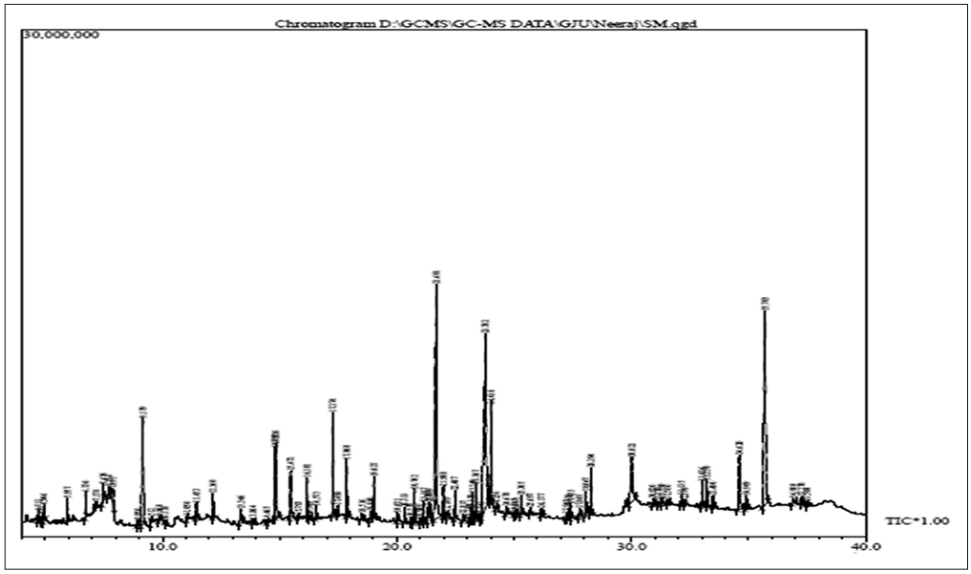

Figure 3: Gas chromatography-mass spectroscopy chromatogram of the constituents of methanol extract of seeds of *Brassica oleracea*

acid glyceryl 2 linoleate, and (Z,z)-6,9-cis-3,4-epoxy-nonadecadiene its esters. Furthermore, some phytosterols such as stigmasterol and gamma-sitosterol were present in significant quantity. In addition to all above compounds, some other compounds were also present which includes Vitamin E, 2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one, diphenylmethanone, pentadecafluorooctanoic acid, and dodecyl ester. Some flavonoids, namely 2,4,5-trimethyl-1,3-dioxolane 2,5-Dimethyl-2,4-dihydroxy-3(2H)-furanon, has also been reported. Broccoli also contains phenolic compound viz. 4-vinylguaia, amino acid L-pyroglutamic acid, a sulphur compound-dimethoxysulfone and a glycoside namely 2,4-dihydroxy- 2,5-dimethyl-3(2H)-furan-3-one was also found in the extract. Two lactones, namely, 4-cyclobutanoic acid and 1,2-cyclopentadiene were also present in the extract. One diterpene alcohol 2-hexadecen-1-ol, 3,7,11,15-tetramethyl and two triterpenes Stigmasta-5,22-dien-3-ol, acetate, (3.beta.) and Ergost-5-en-3-ol, (3.beta.,24r)- were also present.

DISCUSSION

Herbs play a vital role as antioxidant against the free radicals formed in various biological processes inside the human body. These antioxidants combine with the reactive oxygen species and terminate the chain reaction before the vital cellular components are damaged. The principal secondary metabolites such as flavonoids, polyphenols, tannins, and saponins are responsible for the antioxidant property of herbs. To ascertain the antioxidant potential of *B. oleracea,* these secondary metabolites have been studied qualitatively as well as quantitatively and also there antioxidant potential was assessed by *in vitro* antioxidant assays. The preliminary phytochemical study confirmed the presence of terpenoids, alkaloids, tannins, steroids, and saponins in the methanol extract of *B. oleracea*. The GC-MS analysis of *B. oleracea* flower extract revealed the presence of 25 compounds having many therapeutic effects. As per the GC-MS results, *B. oleracea* has many antioxidant compounds likewise 4-vinylguaia (0.88%), myristic acid and ethyl ester (1.29), ascorbic acid 2,6-dihexadecanoate (14.68%), heptadecanoic acid (0.70 %), Vitamin E (0.56), stigmasterol (0.74%), and hexadecanoic acid and 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.09%). Stigmasterol an unsaturated phytosterol is a precursor of progesterone, which is helpful in tissue rebuilding mechanisms related to estrogen effects, and also acts as an precursor of Vitamin D3 and intermediate in the biosynthesis of androgens, estrogens, and corticoids.[34] Plant also has anti-inflammatory compounds such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (5.46%), and 2-methoxy-4-vinylphenol (0.88%) and few antitumor agents such as delta (2)-angelica lactone (0.42%), tetradecanoic acid and ethyl ester (1.29%), octadecenoic acid and ethyl

Table 2: Phytoconstituents identified along with their RIs, nature, and pharmacological action

Rt	RI	Percentage area	Name	Synonyms	Molecular formula	Molecular weight	Nature of compound	Pharmacological action	Reference
4.712	917	0.34	4-Cyclobutanoic acid	Dihydro-2 (3 h)-furanone Gamma butyrolactone	C4H7ClO2	122	Lactone	Anti-inflammatory activity, analgesic, ulcerogenic	[17]
4.946	929	0.42	1,2-cyclopentanedione	Delta.(2)-angelica lactone	C5H6O2	98	Carbohydrate	Prevented gastrointestinal tumor growth	[18]
5.957	984	0.48	2,4-Dihydroxy-2,5-dimethyl -3 (2H)-furan-3-one	pyrone	C6H8O4	144	Glycoside	Antifungal and anti-insectant	[19]
7.476	1063	0.51	S-Methyl methanethiosulphonate	Dimethoxysulfone	C2H6O2S2	126	Organosulfur	Suppresses chromosome abberations, antimutagenic agent	[20]
9.179	1151	5.46	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	2,4,5-Trimethyl-1,3-dioxolane 2,5-Dimethyl-2,4-dihydroxy -3 (2H)-furanon	C6H8O4	144	Flavonoid fraction	Anti-inflammatory, analgesic, antibacterial, antifungal	[21]
12.160	1314	0.88	2-methoxy-4-vinylphenol	4-vinylguaia Col	C9H10O2	150	Phenolic compound	Antioxidant antimicrobial anti-inflammatory	[22]
13.366	1385	0.89	DL-Proline, 5-oxo-, methyl ester	L-Pyroglutamic acid	C6H9NO3	143	Natural amino acid	For mental fatigue and memory improvement	[23]
15.471	1515	1.71	2-Hydroxy-1 -(1'-pyrrolidiyl)-1-buten-3-one	Menthone-d1	C8H13NO2	155	Alcohol	Antiseptic, anesthetic, local pain, pruritis, allergic dermetitis	[24]
17.274	1637	2.78	Diphenylmethanone	Trioxolane	C13H10O	182	Ozonoides	Antiviral and antimicrobial	[25]
17.868	1678	1.72	Pentadecafluorooctanoic acid, dodecyl ester	Cetylpyridinium chloride	C20H25F15O2	582	Cationic quaternary ammonium compound	Antibacterial	[26]
19.027	1762	1.29	Tetradecanoic acid, ethyl ester	Myristic acid, ethyl ester	C14H28O2	228	Fatty acid	Antioxidant, cancer preventive	[27]
20.762	1893	1.00	Octadecenoic acid, ethyl ester	Ethyl oleate	C14H22N2O	234	Fatty acid ester	Hemolytic agent cancer preventive	[27]
21.691	1968	14.68	Hexadecanoic acid, methyl ester	Ascorbic acid 2,6-dihexadecanoate	C16H32O2	256	Palmitic acid ester	Antioxidant	[28]
22.487	2034	0.70	Heptadecanoic acid	Ethyl margarate	C17H34O2	270	Fatty acid	Antioxidant	[27]

(Contd...)

Table 2: *(Continued)*

Rt	RI	Percentage area	Name	Synonyms	Molecular formula	Molecular weight	Nature of compound	Pharmacological action	Reference
23.387	2110	1.38	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]-	Phytol	C20H40O	296	Diterpene alcohol	Anti-inflammatory	[8]
23.782	2145	15.39	(Z, z)-6,9-cis-3,4-epoxy -nonadecadiene	Linoleic acid chloride	C19H34O	278	-	No activity reported	[29]
24.018	2165	2.66	Octadecanoic acid	Stearic acid	C18H36O2	284	Polyenoic fatty acid	Decreases plasma cholesterol	[30]
28.067	2508	1.09	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2-Monopalmitoylglycerol	C19H38O4	330 902	Amino compound	Hemolytic, pesticide, flavor, antioxidant	[31]
30.021	2599	3.49	Nonanoic acid, 9-(3-hexenylidenecyclopropylidene	Glyceryl2 linoleate	C21H36O4	352	Fatty acid ester	Cosmetic, coloring agent	[31]
33.026	3138	0.83	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)	Stigmasterol acetate	C31H50O2	454	Triterpene	Antihepatotoxic, anti-inflammmatory, antiophidic, antioxidant, artemecide, extrogenic, sedative	[15]
33.230	3149	0.92	1,2-hexadecanediol	ARACHIDIC alcohol	C16H34O2	258	Alcohol compound	Nf	-
33.490	3163	2366773	Vitamin E	Alpha.-tocopherol	C29H50O2	430	Organic compound	Antiaging, analgesic, antidiabatic anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, ypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary	[21]
34.620	3240	3.16	Ergost-5-en-3-ol, (3.beta.,24r)-	Stigmasterol acetate	C28H48O	400	Phytosterol	Dyslipidemia and cardiovascular	[32]

(Contd...)

Table 2: *(Continued)*

Rt	RI	Percentage area	Name	Synonyms	Molecular formula	Molecular weight	Nature of compound	Pharmacological action	Reference
34.909	3265	0.74	Stigmasterol	Chondrillasterol	C29H48O	412	Phytosterol	Antioxidant, hypoglycemic and thyroid -inhibiting properties, precursor of progesterone, antimicrobial, anticancer, anti-arthritic, anti- asthma, anti-inflammatory, diuretic	[31]
35.703	3334	12.45	Gamma-sitosterol	25-homo-24-ketocholesterol	C29H50O	414	Phytosterol	Reduces hyperglycemia	[33]

RIs: Retention indices, Rt: Retention time

ester (1.00%), and Vitamin E (0.56%). Some antifungal, antibacterial, and antiviral compounds have also been found, namely 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (0.48%), 2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one (1.71%), trioxolane (2.78%), pentadecafluorooctanoic acid, and dodecyl ester (1.72%). Few antidiabetic/hypoglycemic compounds have also been reported such as Vitamin E (0.56%), Stigmasterol (0.74%), Gamma-sitosterol (12.45%), hypocholesterolgenic agent ergost-5-en-3-ol, (3.beta., 24r)- (3.16%), octadecanoic acid (2.66%), and an organosulfur compound dimethoxysulfone (0.51%) having antimutagenic property which is also present in the extract. Plant extract also has a natural amino acid, namely L-pyroglutamic acid, which has been reported to be useful in memory improvement and mental health.

CONCLUSION

The present study demonstrates that methanol extract of *B. oleracea* has a significant antioxidant potential. Furthermore, it has many therapeutically active constituents which can be of great importance for the pharmaceutical industry. These compounds should be isolated and explored further for their medicinal use.

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