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GAS CHROMATOGRAPHY MASS SPECTROMETRY PROFILING, PHARMACOLOGICAL ACTIVITIES OF ETHANOL FLOWER EXTRACT OF *BRASSICA OLERACEA* VAR. ITALICA-BROCCOLI

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ABSTRACT

There are over a two lakh twenty thousand phytochemicals out of which fruits and vegetables have hundred different phytochemicals and two hundred and fifty thousand species of plants exists on this planet containing different kinds of secondary metabolites, known as Phytochemicals.Natural antioxidants having low or no side effects are used in prevention of diseases plants produce secondary metabolites, most of the antioxidant compounds are flavonoids, phenols ,anthocyanins, carotenoids are used to overcome the oxidative stress caused by reactive oxygen species.Current research studies were carried out for evaluating the antioxidant and antibacterial activities of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli. Antioxidant activities such as $DPPH^{\cdot}$ radical, Superoxide ($O_2^{\cdot-}$) radical, $ABTS^{\bullet+}$ radical cation, phosphomolybdenum reduction and Fe^{3+} reduction were carried out for ethanol flower extract of *Brassica oleracea* var. italica-Broccoli. The maximum $DPPH^{\cdot}$ radical and Superoxide ($O_2^{\cdot-}$) radical scavenging activities were 54.39±0.42% and 73.07±0.29% at 120 µg/mL concentration and the IC_{50} values were 80.67 µg/mL and 57.59 µg/mL concentrations respectively. The maximum $ABTS^{\bullet+}$ radical cation scavenging activity was 87.63±0.42% at 12 µg/mL concentration and the IC_{50} value was 6.50 µg/mL concentration respectively. The maximum Mo^{6+} reduction and Fe^{3+} reduction were 90±0.26% and 52±0.31% at 120 µg/mL concentration and the RC_{50} values were 15.15 µg/mL and 104.16 µg/mL concentrations respectively. The antibacterial activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli showed maximum zone of inhibition of 23 mm for *Escherichia coli* at 750 µg/mL concentration. Oleic acid, Quinoxaline, 2-isopropyl-3-phenyl, 4-oxide, n-Hexadecanoic acid were found to be the active compounds detected from GCMS analysis.

KEYWORDS: Superoxide ($O_2^{\cdot-}$) radical, $ABTS^{\bullet+}$ radical cation, Phytochemicals, GCMS.

INTRODUCTION

There is a strong correlation of antioxidant consumption with lower risk of many diseases such as cardiovascular cancer, diabetes and hypertension diseases as well as other medical conditions. Vegetables have phenolic compounds, pigments and natural antioxidants; these compounds protect many diseases like cancer and heart diseases.[1,2] The importance of antioxidant effects on cardiovascular diseases and cancer is especially important and these antioxidants can be found in various fruits, vegetables and herbs.

Antioxidants in fruits and vegetables have defensive effects and are three main groups: vitamins, phenolics and carotenoids. Vitamin C (ascorbic acid, AminoAcids) and the oxidized form (dehydroascorbic acid, DHAA), carotenoids and phenolic compounds prevent cardiovascular disease, cancer and cataracts which are associated with the oxidative damage of lipids, DNA and proteins. Moreover, some carotenoids also have antioxidant activity (AOA) and shown beneficial effects on the reduction of cardiovascular diseases. The vegetables that have phytochemicals are also not only low in fat and saturated fat, cholesterol and calories but also are rich in potassium and sodium, fiber, folic acid and AA.[3,4] Broccoli - *Brassica oleracea* L. var. italica Plenk - belongs to family Brassicaceae (Figure 1). The word "*Brassica*" means to cut off the head. Broccoli is an Italian word from the Latin brachium, meaning an arm or branch. The term sprouting as used in sprouting broccoli refers to the branching habit of this type, the young edible inflorescences often being referred to as sprouts. The sprouting broccolis are thought to have originated from the eastern Mediterranean then introduced into Italy.

Broccoli inflorescence is a good source of health promoting compounds since it contains glucosinolates,

flavonoids, hydroxycinnamic acids and other minor compounds.[5] Broccoli is a rich source of carbohydrates, potassium, vitamin K, vitamin C, vitamin A, vitamin E, potassium and folate. It is a very good source of dietary fiber, protein, calcium, phosphorus, magnesium and sodium.[6] The consumption of broccoli may alter the stannous dichloride toxicity. Broccoli extract may use as a new protective strategies against the toxic effect of $SnCl_2$ on patients who were taken Technetium-99m.[7]

Processes such as blanching, cooking and cutting, affect the content of glucosinolates, sulforaphane, polyphenols and antioxidant activity in broccoli. Steam processed broccoli should have enhanced antioxidant (through phenols and flavonoids) and anticarcinogenic (through glucosinolates) properties. Cutting or chopping broccoli then cooking or blanching at temperatures lower than 100°C is favorable for anticarcinogenic properties. These issues seem promising for the use of processed broccoli as a functional food, with improved health promoting properties.[8] Steam processed broccoli showed a higher antioxidant capability, due to the significantly increased extractability of phenols and flavonoids, increased the bioavailability of these compounds in vivo, thus improving the health promoting properties.[9,10,11]

Taxonomic Classification of *Brassica oleracea* var. italica

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Binomial name: *Brassica oleracea var. italic*

Fig. 1: Habitat of *Brassica oleracea* var. italic.

MATERIALS AND METHODS

Collection and Extraction process of *Brassica oleracea* var. italica-Broccoli flowers

The *Brassica oleracea* var. italic (Broccoli) were freshly collected from Koyambedu vegetable market, Chennai, Tamil Nadu, India. The stalk of Broccoli were completely removed and the flowers of Broccoli were well cleaned, made into fine medium pieces and soaked in ethanol for 72 hours. The intense green coloured supernatant was filtered and condensed in room temperature devoid of heat supply, which yields green pasty extract.[12,13]

In vitro antioxidant activities

DPPH˙ radical scavenging activity

The radical scavenging activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was carried out by the reduction DPPH˙ free radical method.[14] One mL of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli with various concentrations (20-120 µg/mL) was mixed with 1 mL of 0.1 mM DPPH solution in methanol. The mixture was then allowed to stand for 30 min incubation in dark. One mL of methanol mixed with 1 mL of DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference.

The percentage of inhibition was calculated as.

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Superoxide ($O_2^{\cdot}$-) radical scavenging activity

Superoxide ($O_2^{\cdot}$-) radical scavenging activity was carried out by the method[15] and the reaction mixture contains different concentrations (20-120 µg/mL) of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli with 50 mM of phosphate buffer (pH 7.4), 200 µL of 1.5 mM of riboflavin, 200 µL 12 mM of EDTA and 100 µL 50 mM of NBT, added in that sequence. The reaction

was started by illuminating the reaction mixture for 15 min in UV lamp. After illumination, the absorbance was measured at 590 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as.

$$\% \text{ of Superoxide } (O_2\text{-}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

ABTS$^{\bullet+}$ (2,2-azinobis (3-ethylbenzo thiazoline-6-sulfonic acid) radical cation scavenging activity

The ethanol flower extract of *Brassica oleracea* var. italica-Broccoli from the stock solution was taken in various concentrations and this assay was performed according to the method.[16] The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. Fresh ABTS solution was prepared for each experiment. The ethanol flower extract of *Brassica oleracea* var. italica-Broccoli in varying concentrations (2-12 µg/mL) were allowed to react with 500 µL of the ABTS solution for 15 minutes in dark condition and the absorbance was measured at 734 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as.

$$\% \text{ of ABTS}^{\bullet+} \text{ radical cation inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Phosphomolybdenum reduction activity

The antioxidant capacity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was assessed as described.[17] The ethanol flower extract of *Brassica oleracea* var. italica-Broccoli with varying concentrations ranging (20-120 µg/mL) was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 95^{o}C for 90 min. The absorbance of the coloured complex was measured at 695 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as.

$$\% \text{ of Phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

Ferric (Fe^{3+}) reducing power activity

The reducing power of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was determined by slightly modified method.[18] One mL of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli in different concentrations (20-120 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1 % w/v). The mixtures were then incubated at 50°C for 20 min in water bath. 500 µL of trichloroacetic acid (10 % w/v) was added to each mixture, followed by 100 µL of Ferric chloride (0.01%, w/v) was added and the absorbance was measured at 700 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as.

$$\% \text{ of } Fe^{3+} \text{reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

Determination of total phenols

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds[19] with slight modifications. One hundred µL of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli (1 mg/mL) was mixed with 900 µL of methanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of Na_2CO_3 (20% w/v) was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured using UV-Vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

Determination of total flavonoids

The total flavonoid content of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was determined using aluminium chloride colorimetric method with slight modification as described.[20] 500 µL of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli (1 mg/mL) was mixed with 500 µL of methanol, 0.5 mL of 5% (w/v) sodium nitrite solution and incubated for 5 min at room temperature. Then, 0.5 mL of 10% (w/v) aluminium chloride solution was added and incubated for further 5 min at room temperature followed by addition of 100 µL of 1 M NaOH solution. The total volume was made up to 2 mL with distilled water. The absorbance was measured at 510 nm using UV-Vis spectrophotometer. The total flavonoid content was expressed in terms of quercetin equivalent (µg/mg of extract), which is a common reference compound.

Antibacterial activity by Agar well diffusion method

Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24 hours grown bacterial pathogens were swabbed on nutrient agar plates.[21] Then, the ethanol flower extract of *Brassica oleracea* var. italica-Broccoli in varying concentrations (250 µg/mL, 375 µg/mL, 500 µg/mL and 750 µg/mL) was loaded in the clean lawns made using sterile cork borer. Tetracycline (30 µg) was used as standard. The plates were then incubated at 37ºC for 24 hours and after incubation, the inhibition diameter was measured and recorded.

Gas chromatography–Mass Spectrometry (GC–MS) analysis

For GC-MS analysis, the ethanol flower extract of *Brassica oleracea* var. italica-Broccoli were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode.[22] Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units.

RESULTS AND DISCUSSION

DPPH$^{\cdot}$ radical and Superoxide ($O_2^{\cdot-}$) radical scavenging activities of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli

Evaluation of antioxidant activity by DPPH method is the best screening option for herbal based drugs. DPPH$^{\cdot}$ (1,1-Diphenyl-2-picrylhydrazyl) is a stable nitrogen centered free radical which has an unpaired valence electron at one atom of nitrogen bridge.[23] The ability of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picryl hydrazyl radical (DPPH). The ethanol flower extract of *Brassica oleracea* var. italica-Broccoli demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picryl hydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picryl hydrazine and the reducing capacity increased with increasing concentration of the extract. The maximum DPPH$^{\cdot}$ radical scavenging activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was 54.39±0.42% at 120 µg/mL concentration (Table 1). The IC_{50} value for the ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was found to be 80.67 µg/mL concentration respectively (Figure 2) and was compared with standard (Ascorbic acid, IC_{50} = 13.81 µg/mL concentration). The ethanolic extract exhibited higher antioxidant activity in DPPH radical and superoxide anion scavenging than that of aqueous extract.[24]

Superoxide anion is also very harmful to cellular components and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to blue formazan, which is measured at 590 nm using UV-Vis spectrophotometer. Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture.[25] The maximum superoxide ($O_2^{\cdot-}$) radical scavenging activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was 73.07±0.29% at 120 µg/mL concentration (Table 1 and Figure 2) and the IC_{50} value for the ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was found to be 57.59 µg/mL concentration respectively. It was compared with the standard of ascorbic acid (IC_{50} = 16.47 µg/mL concentration).

$ABTS^{\bullet+}$ radical cation scavenging activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli

$ABTS^{\bullet+}$ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and $ABTS^{\bullet+}$ radical cation gets reduced in the presence of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli and the remaining radical cation concentration was then quantified at 734 nm. It can be prepared using $K_2S_2O_8$ as an oxidant. The blue-green colour of ABTS solution is formed by the loss of an electron by the nitrogen atom of ABTS (2, 2-azinobis (3ethylbenzothiazolin-6-sulfonic acid)). The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom quenches the hydrogen atom.[26] The maximum $ABTS^{\bullet+}$ radical cation scavenging activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was 87.63±0.42% at 12 µg/mL concentration (Table 2 and Figure 2) and the IC_{50} value for the ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was found to be as 6.50 µg/mL concentration respectively, which was compared with standard ascorbic acid (IC_{50} = 4.59 µg/mL concentration).

Phosphomolybdenum reduction and Ferric (Fe^{3+}) reducing power activities of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli

The total antioxidant activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was measured spectrophotometrically by phosphomolybdenum reduction method, which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm.[27] The maximum phosphomolybdenum reduction of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was 90±0.26% at 120 µg/mL concentration with the RC_{50} value of 15.15 µg/mL concentration respectively (Table 1 and Figure 2). It was compared with the standard ascorbic acid (RC_{50} = 7.15 µg/mL).

The reducing power of Fe^{3+} to Fe^{2+} by ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was studied and showed reduction ability in a dose dependent manner. The maximum reduction of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli was 52±0.31% at 120 µg/mL concentration (Table 1 and Figure 2). Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action.[28] The RC_{50} value for the ethanol flower extract of *Brassica oleracea* var. italica-Broccoli as found to be 104.16 µg/mL

concentration respectively and was compared with the standard (21.68 µg/mL concentration) Ascorbic acid.

Table 1: *In vitro* antioxidant activities of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli.

S.No	Concentration (µg/mL)	% of inhibition*		% of reduction*	
		DPPH˙ radical	Superoxide ($O_2^{\bullet-}$) radical	Mo^{6+} reduction	Fe^{3+} reduction
1	20	26.17±0.15	33.2±0.10	66±0.22	36±0.44
2	40	30.3±0.26	37.06±0.12	70±0.17	41±0.19
3	60	42.27±0.35	52.09±0.37	78±0.28	44±0.10
4	80	49.58±0.11	60.13±0.18	80±0.13	46±0.24
5	100	52.01±0.46	70.1±0.33	82±0.48	48±0.37
6	120	54.39±0.42	73.07±0.29	90±0.26	52±0.31

(*Average value of 3 replicates)

Table 2: $ABTS^{\bullet+}$ radical cation scavenging activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli.

S.No	Concentration (µg/mL)	% of inhibition*
		$ABTS^{\bullet+}$ radical cation
1	2	22.35±0.28
2	4	35.47±0.19
3	6	46.12±0.36
4	8	68.09±0.21
5	10	83.52±0.27
6	12	87.63±0.42

(*Average value of 3 replicates)

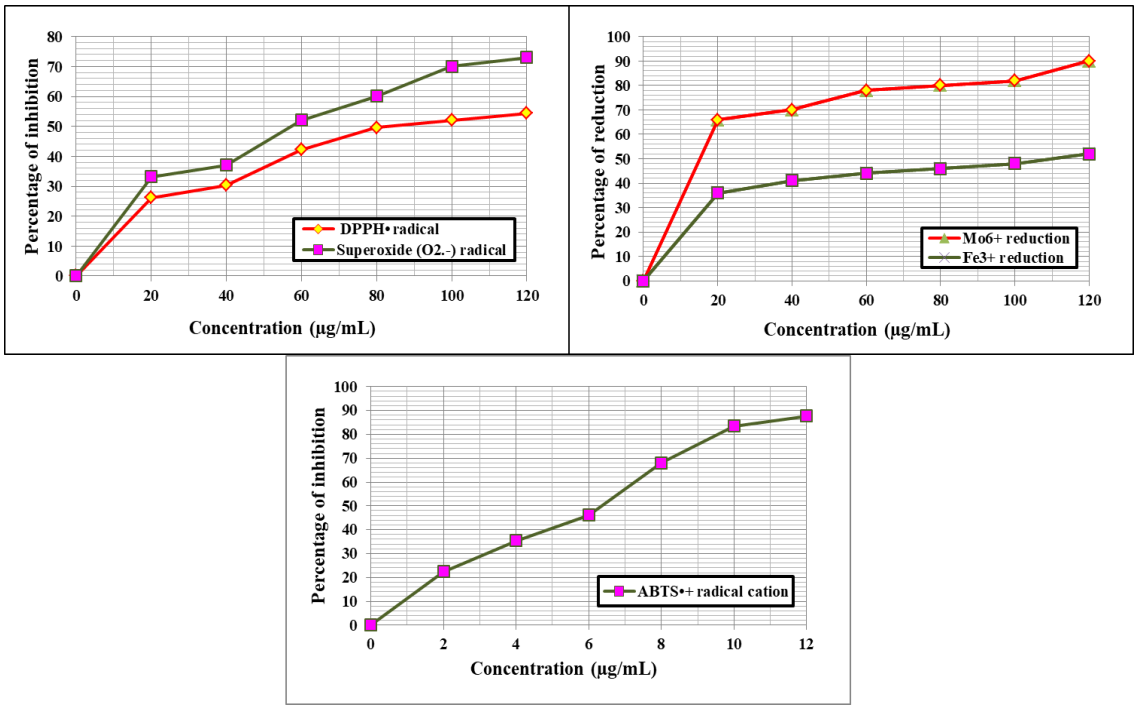


Fig. 2: *In vitro* antioxidant activities of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli.

Determination of total phenols and flavonoids

Flavonoids and phenolics acids are the most important bioactive natural product of secondary metabolites and act as an antioxidant and anti-aging substances, capable of scavenging free radicals and reducing the risk of cancer.[29] Oxidative stress is a harmful condition that occurs when there is an excess of ROS and decrease in antioxidant levels and cause tissue damage which leads to different diseases. Flavonoids and phenolic compounds are well known for their antioxidant activity that protect humans against the damaging effects of free radicals in addition an imbalance between antioxidants and free radicals results in oxidative stress, will lead to cellular damage. Phenolic hydroxyl groups are good hydrogen donors, which are hydrogen-donating antioxidants can react with reactive oxygen species and reactive nitrogen species which breaks down the generation of new radicals in a termination reaction.

Phenolic structures often have the potential to interact strongly with proteins, due to their hydrophobic benzenoid rings and hydrogen-bonding potential of the phenolic hydroxyl groups. Phenolic compounds have the ability to act as antioxidants also by virtue of their capacity to inhibit some enzymes involved in radical generation, such as various cytochrome P450 isoforms, lipoxygenases, cyclooxygenase and xanthine oxidase.[30]

The total phenol content was 251.57±0.23 µg/mg of GAE and the total flavonoid content was 38.60±0.34 µg/mg of QE in the extract (Table 3 and Figure 3). These results provide a comprehensive profile of the antioxidant activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli with respect to their phenols and flavonoids content.

Table 3: Qualitative phytochemical analysis of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli.

S.No	Phytochemicals	Tests	Results
1	Alkaloids	(a)Mayer's test	+
		(b) Hager's test	+
2	Phenols	Ferric chloride test	+
3	Tannins	Lead acetate test	+
4	Flavonoids	Sodium hydroxide test	+
5	Glycosides	Legal's test	+
6	Steroids	Libermann-Burchard test	+
7	Terpenoids	Salkowski test	+
8	Saponins	Foam test	-

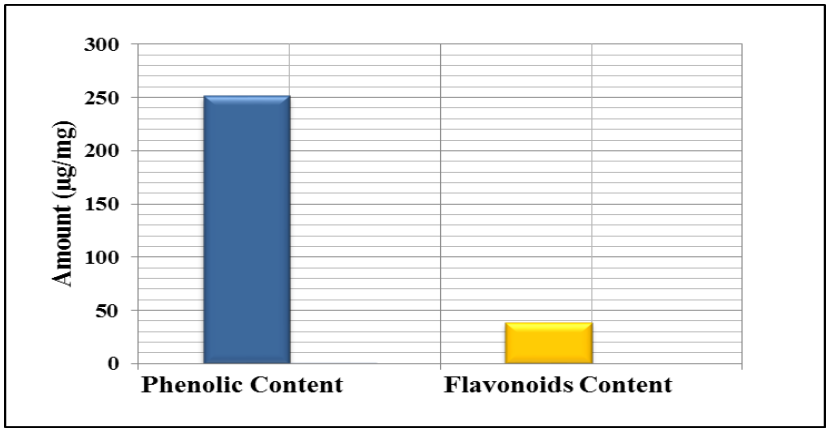


Fig. 3: Determination of total phenols and flavonoids content of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli.

Antibacterial activity by Agar well diffusion method

The ethanol flower extract of *Brassica oleracea* var. italica-Broccoli were investigated for *in vitro* antibacterial activity against microorganisms including Gram-positive bacteria (*Micrococcus luteus*, *Bacillus subtilis*) and Gram-negative bacteria *(Proteus vulgaris*, *Shigella flexneri*, *Escherichia coli*). The antibacterial sensitivity of the ethanol flower extract of *Brassica oleracea* var. italica-Broccoli and their potency were assessed quantitatively by measuring the inhibitory zone around the wells in the petriplates (Table 4).

Table 4: Antibacterial activity of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli.

S.No	Bacterial pathogens	Zone of inhibition (mm)				
		Standard Tetracycline – 30 µg	250 µg/mL	375 µg/mL	500 µg/mL	750 µg/mL
1	*Bacillus subtilis*	11	13	15	15	21
2	*Micrococcus luteus*	22	10	11	12	14
3	*Shigella flexneri*	13	16	17	20	21
4	*Proteus vulgaris*	12	15	17	17	19
5	*Escherichia coli*	15	19	21	22	23

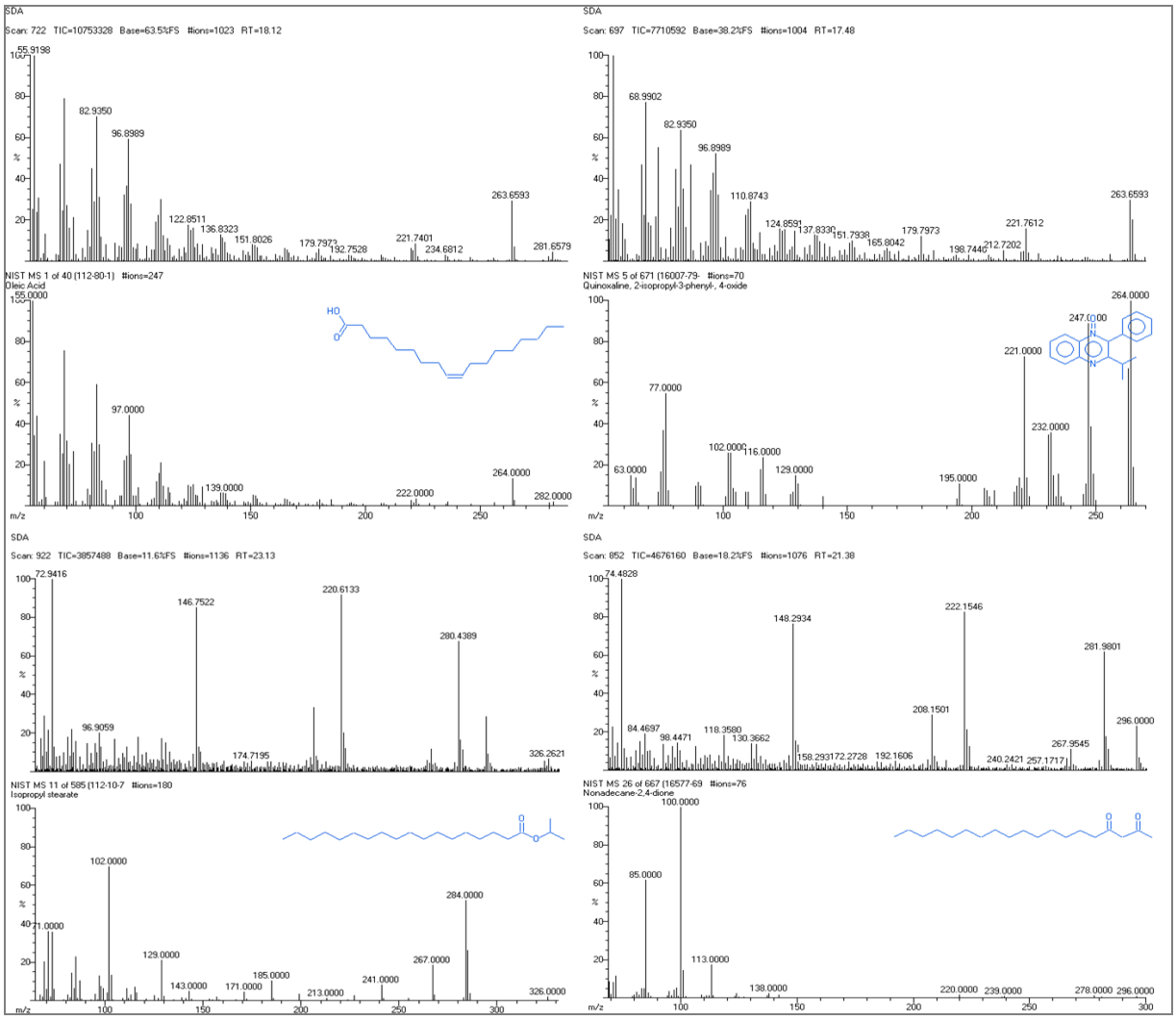
The maximum inhibitory effect for ethanol flower extract of *Brassica oleracea* var. italica-Broccoli against *Escherichia coli* was 23 mm at 750 µg/mL concentration and minimum inhibitory effect against *Micrococcus luteus* was 14 mm at 750 µg/mL concentration respectively. The antibacterial activity of the ethanol flower extract of *Brassica oleracea* var. italica-Broccoli could be correlated as due to the presence of secondary metabolites such as flavonoids, phenolic compounds, terpenoids, tannins and alkaloids that adversely affect the

growth and metabolism of microbes. Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance.[31]

Tannins bind to proline rich proteins and interfere with the protein synthesis.[32] Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls.[33] Coumarins are also known act against gram positive bacteria and it is produced in carrots in response to fungal infection which could be attributed to its antimicrobial activity.[34] Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes.[35]

Gas chromatography–Mass Spectrometry (GC–MS) analysis

The GC-MS analysis of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli (Figure 4) revealed the presence of ten different bioactive compounds (phytochemical constituents) that could contribute the antioxidant and therapeutic benefits of Broccoli. Several mechanisms have been proposed for antioxidant activity due to presence of glucosinolates hydrolysis product (allyl isothiocyanates) as potent inducers of phase II enzymes which are important in the detoxification of electrophiles and protection against oxidative stress.[36] The identification of the phytochemical compounds was confirmed based on the peak area, retention time, molecular weight and molecular formula (Table 5 and Figure 5).



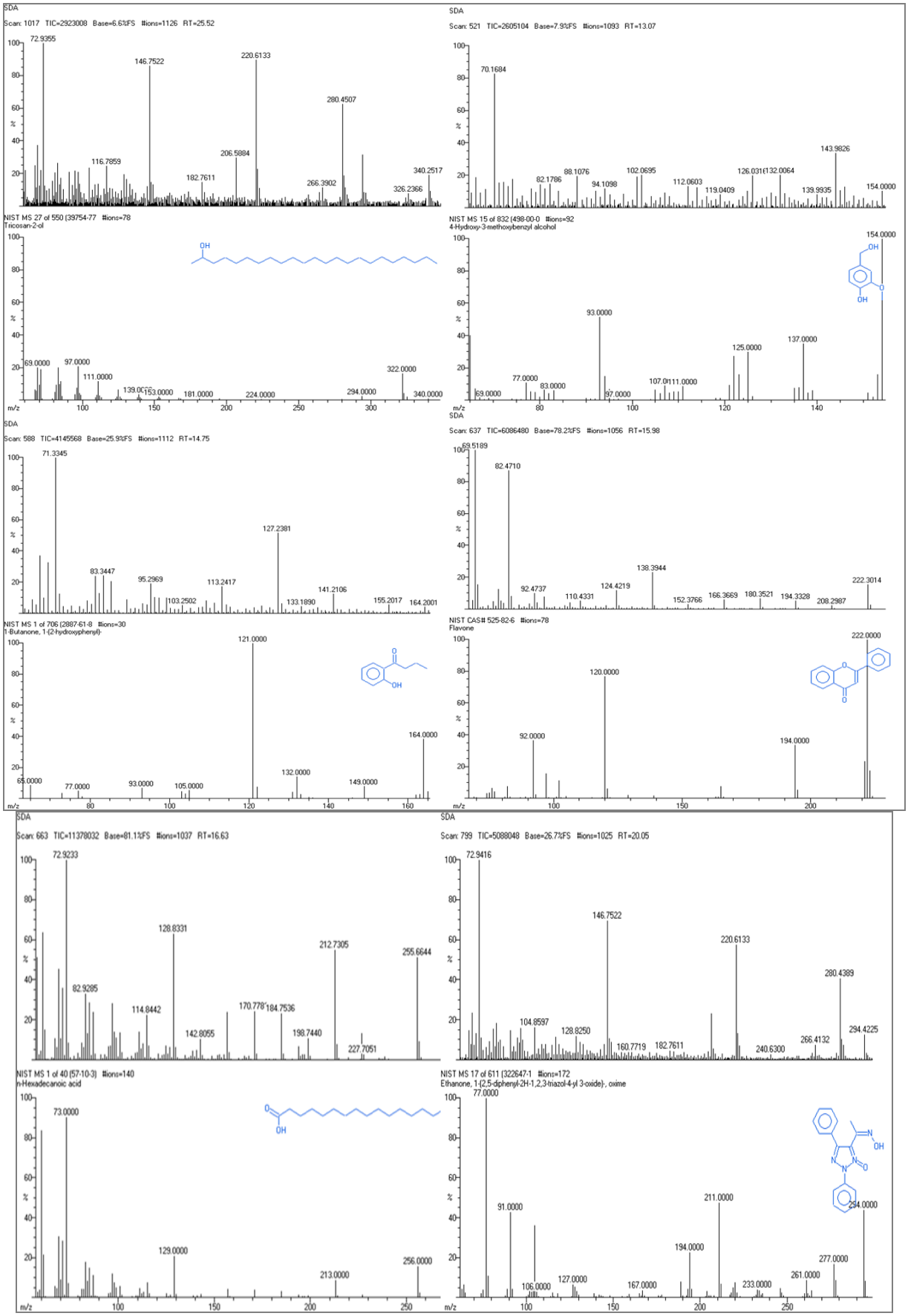


Fig. 4: Mass Spectrum of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli by GCMS analysis.

Table 5: Active pharmacological compounds and properties of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli.

S.No	Compound Name	RT	Molecular weight	Molecular formula	Pharmacological activity[37-40]
1	Oleic acid	18.12	281.65	$C_{18}H_{34}O_2$	5 alpha reductase inhibitor Hypocholestrolemic activity Perfumery and flavour Cancer preventing agent Anti-inflammatory activity Antibacterial activity
2	Quinoxaline,2-isopropyl-3-phenyl, 4-oxide	17.48	263.65	$C_{17}H_{16}N_2O$	Antimicrobial activity Antitubercular activity Antiviral activity Antiprotozoan activity Chronic and metabolic disease bioactivity Chronic inflammation Anti glutameric activity
3	Flavone	15.98	222.30	$C_{15}H_{10}O_2$	Production of Reactive Oxygen Species (ROS) can be reduced by flavonoids Relevance of plant defense mode of action is highly possible by flavonoids Formation of oxygen radicals can be prevented by flavonoids thereby inhibiting the enzyme activity
4	n-Hexadecanoic acid	16.63	255.66	$C_{16}H_{32}O_2$	Antioxidant activity Nematicide and Pesticide Lubricant Antiandrogenic activity Flavor purpose Hemolytic agent

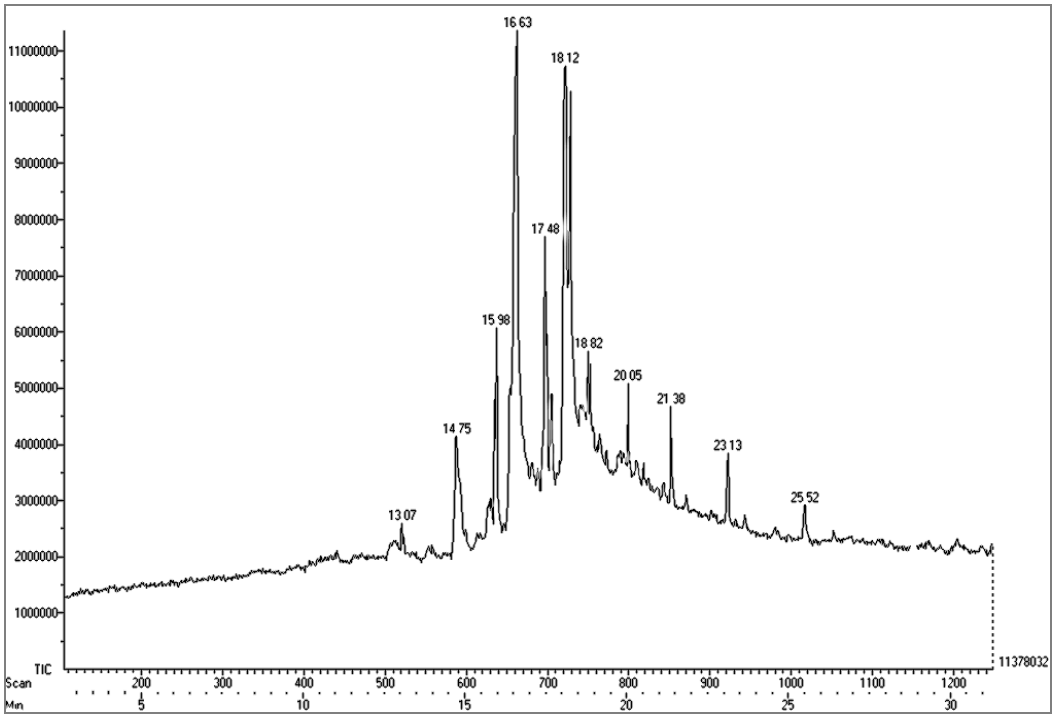


Fig 5: GCMS Chromatogram of ethanol flower extract of *Brassica oleracea* var. italica-Broccoli.

CONCLUSION

Strong recommendations for consumption of natural plant food and the use of nutritional therapy have become progressively popular for health improvement, prophylaxis and treatment of various ailments. The results of the present study provides promising guidelines proving that Broccoli to be the richest source of antioxidant and antimicrobial properties. Hence, the pharmacological mechanism of Broccoli considering at further level shall be evaluated for converting into an active drug. Fruits and vegetables are consumed at all times, and due to their convenient size; they are an excellent between-meal snack. They are relatively low in calories and fat (avocado and olives being the exceptions), they have no cholesterol, they are rich in carbohydrates and fiber, they contain vitamin C and carotene, and some are a good source of vitamin B6. Fruits and vegetables are relatively low in sodium and high in potassium. Ascorbic acid in fruits and vegetables enhances the bioavailability of iron in the diet. Because of all these characteristics, fruits and vegetables have a unique role in a healthy diet.

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