

Comprehensive Analysis of *Brassica oleracea*: Phytochemical Composition, Radical Scavenging, and Anti-Proliferative Activity

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ABSTRACT

Background: Natural sources like plants, vegetables, and fruits contain vast micro and macro nutrients that are useful for livelihood and also act as a medicine for various health conditions. Brassica vegetable naturally contains high antioxidant property which aids in removing free radicals caused by organelles during cellular process. The study aims at preparing *Brassica oleracea* extracts using a range of polar and non-polar solvents and to evaluate its phytochemical, antioxidant and cytotoxicity properties.

Methods: *Brassica oleracea* was extracted using hexane, ethyl acetate and ethanol. All the extracts were subjected to phytochemical analysis and antioxidant activity was performed using DPPH method. The antiproliferative activity was performed on THP-1 cells by MTT assay. The extract showing maximum activity was then characterized using FTIR and GCMS. **Results:** The extract study infers positive results for major secondary metabolites (alkaloids, glycosides, proteins, phenols, tannins, steroids, flavonoids, terpenoids and diterpenes) and negative for quinones and coumarins. DPPH radical scavenging assay showed high antioxidant activity for ethanol extracts 45-91% at 5µg/mL followed by ethyl acetate (37%-80%) and hexane extract (23%-73%). The anti-proliferative activity in THP-1 cells, revealed that the ethanolic extract significantly decreases cell viability relative to hexane and ethyl acetate extracts, indicating its potential as a natural anticancer drug. **Conclusion:** Cytotoxicity studies further demonstrated a concentration dependent effect on cell viability, indicating its potential bioactivity. The structural analysis performed with FTIR and GC-MS revealed important functional groups and bioactive compounds that could play a role in these effects.

Keywords: Antioxidants, Broccoli, DPPH, Flavonoids, FTIR, ROS, THP-1 Cells.

INTRODUCTION

As a source of new drugs and with varying pharmacological potential, natural compounds with a variety of bioactivities are becoming more and more essential. Humans have long utilised plants to treat a variety of illnesses, and they continue to do so today either directly or indirectly as supplements¹. Plants were utilised to treat nearly every illness before the invention of synthetic medications. Several investigations have revealed that plants possess therapeutic qualities, including antimicrobial, analgesic, antiphlogistic, free-radical scavenger, and antineoplastic agents. The occurrence of oxidative stress is due to the discrepancy among the generation of the level of reactive oxygen species (ROS) and the body's capacity to counteract with antioxidant defences. Although reactive oxygen species are crucial for cellular signalling and maintaining homeostasis, an overabundance can harm the proteins, fats, and the DNA molecule, leading to cellular dysfunction. This oxidative damage contributes significantly to the onset of numerous chronic conditions like cancer, cardiovascular disorders, metabolic disorders, as well as neurological disorders such as dementia and Parkinson's diseases². Exposure to environmental toxins, can exacerbate oxidative stress by raising the generation of ROS and lowering the levels of antioxidants³.

To take the place of artificial antioxidants, which are prohibited because of the carcinogenicity,

natural antioxidants have recently drawn a lot more attention for usage in foods or pharmaceutical products⁴. Flavonoids and phenolic acid are secondary metabolites of plants that have been extensively researched for their anti-cancer and antioxidant properties. In many types of vegetables and herbs, phenolic compounds are present, and many of them have antioxidant qualities. Because of this, they can function as reduction agents, oxygen singlet suppressors, and hydrogen donors as well as metal ion chelators⁵. *Brassica oleracea* is a promising natural source of antioxidants, recognised for its powerful bioactive components that may alleviate oxidative stress. *Brassica oleracea* belongs to the family cruciferous is a biennial vegetable originated 2000 years ago from Italy⁶. The vegetable *Brassica oleracea* is largest and widely consumed in Europe and all parts of the world because of their varying class of nutrients. It is considered as an important food crop in India, Japan, China and European countries commonly known as Broccoli. Broccoli is a fast-growing plant, grows about 23-34 inches upright tall and branching, with dense clusters of flowers buds⁷. The green cluster of flowers at the centre of plant is referred to as "head" of broccoli. *Brassica* species are good sources of water soluble and insoluble vitamins and phytochemicals⁸ which will reduce the oxidative damage risk caused by free radicals.

Phytochemical group of *Brassica oleracea* flavonoids, dietary phytoconstituents prevalent antioxidants

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in plants and human diets, are increasingly important due to their antioxidative, estrogenic, antibacterial, anticancer, anti-inflammatory, antimutagenic, myocardial, vasodilator, and hepatoprotective properties⁹. Despite *Brassica oleracea*'s known benefits, limited data exist on its comparative solvent-based extraction efficiency, antioxidant potential, and anti-proliferative activity. The aim of this study is to evaluate the phytochemical composition, antioxidant activity and anti-proliferative effects of different solvent extracts of *Brassica oleracea*, along with characterization using FTIR and GCMS analysis.

MATERIALS AND METHODS

Materials

Plant samples of *Brassica oleracea* (Broccoli) florets were purchased from the supermarket at Guduvanchery, Chengalpet district, Tamil Nadu, India. The samples were authenticated by Siddha Central Research Institute, (B29032301C) Chennai. The THP-1 cell line was purchased from NCCS, Pune, Maharashtra, India. All the chemicals used in this study were of analytical grade and were purchased from Southern India Scientific Corporation, Chennai, Tamil Nadu, India.

Preparation and extraction of the sample

The vegetable sample that was obtained was washed thoroughly using purified water to eliminate the debris. Then the sample was sliced into tiny pieces using a sterile blade and then allowed to dry in the shade for more than two weeks. Sample was coarsely grounded using motor and pestle and stored in an airtight container for further use. *Brassica oleracea* was extracted using hexane (BHE), ethyl acetate (BEAE) and ethanol (BEE) by cold maceration method. For 20g of sample (*Brassica oleracea*) 200mL of solvent was added in a screw capped bottle, in 1:10 ratio. The sample was incubated for 48hrs with constant stirring, filtered by using a Whatman No.1 filter paper, solvent was evaporated by rotavapor method, crude extracts were collected and used for further analysis.

Phytochemical analysis

The phytochemical study of *Brassica oleracea* was carried out to determine whether phenolic chemicals, alkaloids, flavonoids, saponins, steroids, and tannins, terpenoids, coumarins, quinones and diterpenes are present in the crude extracts using test tube methods following the standard protocol prescribed by Upadhyay et al., (2016)¹⁰ and Jahan et al., (2010)¹¹.

Antioxidant activity

The radical scavenging activity of Hexane, ethyl acetate and ethanol extract of *Brassica oleracea* was assayed using 2,2-diphenylpicrylhydrazyl (DPPH) method. It is a radical compound commonly used to evaluate the radical scavenging activity based on electron transfer¹². The experiment was carried out using the methods described by Ligor et al., (2024)¹³ with minor changes. Briefly, 100μL of extract was mixed with 100μL of 0.1mM DPPH solution and incubated for 30 mins in dark. The absorbance was checked at 517nm. The anti-radical activity was calculated using the formula:

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100\%$$

Anti-proliferative activity

The THP-1 cell lines were cultured in DMEM medium with FBS, streptomycin, and penicillin, and raised in a CO₂ incubator at 37°C. The MTT assay is an indicator of cell viability, activity and proliferation of cells by measuring cellular metabolic activity in THP-1 cells. The

cytotoxicity level of THP-1 cells against BHE, BEAE, BEE extracts was studied by a sensitive, reliable and colorimetric method based on the reduction of yellow tetrazolium salt (MTT) to purple formazan crystals by metabolically active NAD(P)H-dependent oxidoreductase enzymes¹⁴. Shortly, 96-well plates were seeded with THP-1 cells at a concentration of 1×10^4 cells/ well in 200μl culture medium and incubated for 1hr. After incubation, cells were treated with 10ml of extract (5mg/ml) followed by 24hr incubation with 10μl of MTT solution along with 5% FBS. Again, the plates were incubated for 3hrs, finally 100μl of DMSO was added and quantified. Insoluble formazan crystals are dissolved by adding solubilization and the absorbance were measured at 500–600nm. Number of live, metabolically active cells increases with darkness of the fluid. Formula to calculate cell inhibition percentage is:

$$\text{Percentage cell inhibition} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control}} \times 100$$

Fourier transform infrared spectroscopy (FTIR)

The functional group analysis of the ethanolic extract of *Brassica oleracea* plant components was conducted using Fourier transform infrared spectroscopy (FTIR) using a Shimadzu IRTracer-100 spectrophotometer with high sensitivity measurements and an SN ratio of 60,000:1¹⁵. An FTIR spectroscope was used to load each sample onto a plate and run it at a resolution of 4 cm⁻¹ over an infrared wave number range of 500 to 4000 cm⁻¹. The functional categories of secondary metabolites in each milligram of dried sample may have the ability to scavenge free radicals by compressing the sample with 100 milligrams of potassium bromide (KBr).

Gas chromatography mass spectrometry (GC-MS) analysis

Brassica oleracea ethanolic extract was analysed for compound detection by GCMS. The sample was injected into Shimadzu, QP2010 SE single quadrupole GC-mass spectrometer in a column oven temperature 50°C during initial up till 280°C for final hold, injection temperature 250°C, total flow 54mL/min, hold time 2mins. Helium (99.95%) carrier gas was employed at a flow rate of 1mL/min¹⁶, the initial oven temperature was set at 110°C and spectral masses were measured at 70 eV. For analysis one milligram of crude extract dissolved in suitable solvent to obtain a clear solution without any particles in it and injected into the splitless mode with a temperature set at 250°C.

RESULTS AND DISCUSSION

Phytochemical analysis

All three extracts of *Brassica oleracea* showed positive test results for phenols, flavonoids, alkaloids, tannins, terpenoids, glycosides, diterpenes and proteins. Steroids test positive for Hexane and Ethyl acetate and negative for ethanol. Coumarins and quinones are negative for all the three samples (Table 1). Phytochemicals are secondary metabolites which is produced mainly by plants, bacteria and fungi. They are low molecular weight molecules having diverse biological activities and chemical structures¹⁷. These metabolites help in plant growth, morphological differentiation and protecting the plants against various environmental constraints. Primary phytochemicals like phenols, alkaloids, proteins and glycosides play a major role in biological activity. Alkaloids compound contains nitrogen atom in a heterocyclic ring structure exhibiting antioxidant and antimicrobial activity. Among the phytochemicals group, phenols possess antioxidant activity containing large number of compounds with nearly greater than 8,000 compounds characterized by one aromatic ring with one or more hydroxyl groups attached.

Table 1: Phytochemical analysis of *Brassica oleracea*.

Test	Samples		
	BHE	BEAE	BEE
Alkaloids	+	+	+
Glycosides	+	+	+
Proteins	+	+	+
Terpenoids	-	-	-
Phenols and Tannins	+	+	+
Steroids	+	+	-
Flavonoids	+	+	+
Terpenoids	+	+	+
Coumarins	-	-	-
Quinones	-	-	-
Diterpenes	+	+	+

“+” indicates presence and “-” indicates absence of the particular phytoconstituents.

DPPH radical scavenging activity

It is one of the widely preferred and most employed methods for measuring radical scavenging activity in plant extracts. This is simple, standardized and relatively rapid method. DPPH is a nitrogen-centered, stable free radical that turns yellow to violet during a reduction process. Color change is dependent on the extract scavenging ability by donating a hydrogen atom. For every assay, ascorbic acid served as the positive control. All the 3 extracts showed strong radical scavenging activities in a concentration dependent manner. Scavenging activity was demonstrated by hexane extract at a variety of 23%-73%, Ethyl acetate extracts showed 37%-80%, Ethanol extracts showed 45% - 91% scavenging activity (Figure 1). These compounds exhibited concentration-dependent antioxidant properties that were significant ($p < 0.01$), greater the concentration levels raise the exhibition of activity related to radical scavenging. This was comparable to the 91%-95% antioxidant activity of the positive control ascorbic acid. Among the three tested extracts the ethanol extract showed the highest antioxidant activity. This is consistent with an earlier study showing that ethanol serves as a strong solvent for the extraction of phenolic compounds and flavonoids from *Brassica* species, which play a major role in their antioxidant properties¹⁸. This can be attributed to the existence of bioactive compounds that include flavonoids, Vit C, glucosinolates, polyphenols, quercetin and kaempferol which are more effectively extracted in polar solvents¹⁹. This highlights the potential application of *Brassica oleracea* in functional food and pharmaceutical formulations aimed at combating oxidative stress-related disorders.

Anti-proliferative study of extracts

The anti-proliferative effects of extracts from *Brassica oleracea* were evaluated on THP-1 cell lines by measuring the proportion of viable cells after being treated with different solvent extracts. The findings (Figure 2) demonstrate a decrease in cell viability for all tested extracts in contrast with the control group. Among the extracts, the ethanolic extract (BEE) showed the most significant reduction in cell viability ($96.48\% \pm 3.51$), followed closely by the ethyl acetate extract (BEAE) ($97.72\% \pm 0.20$) and the hexane extract (BHE) ($98.26\% \pm 0.72$). The decrease in viability indicates that the phytochemicals found in *Brassica oleracea* extracts, such as flavonoids, glucosinolates, and polyphenols, may be responsible for the cytotoxic effects on THP-1 cells. Ethanol, being a polar solvent, tends to extract a higher abundance of bioactive compounds, which could clarify the more significant decline in cell viability seen with BEE compared to the other extracts. Likewise, the moderate cytotoxicity noted in BEAE may be linked to medium-polarity phytochemicals like flavonoids and specific glucosinolates. The hexane extract (BHE), having the lowest polarity, presented the

smallest reduction in viability, probably due to the extraction of mainly non-polar compounds that have limited anti-proliferative effects¹⁸.

The possible mechanisms behind the observed anti-proliferative effects might include the production of reactive oxygen species (ROS), an interruption of cell cycle, or the induction of programmed cell death. Prior research has indicated that phytochemicals derived from *Brassica*, especially glucosinolates and their hydrolysis products (isothiocyanates), can trigger apoptosis and cytotoxicity mediated by oxidative stress in cancer cells²⁰. Additionally, the antioxidant properties of these extracts might affect cellular redox balance, thus influencing proliferation¹⁹. The results are consistent with earlier studies that have shown the anti-proliferative actions of compounds from broccoli. For example, methanolic extract of *Brassica* vegetables, has been documented to cause dose-dependent cytotoxic effects in lung adenocarcinoma cells²¹. Furthermore, the variations in cytotoxicity among the solvent extracts imply that the polarity of the solvent plays a vital role in extracting bioactive compounds with differing levels of effectiveness²².

FTIR

Ethanol extract of *Brassica oleracea* FTIR analysis spectra results are displayed in (Figure 3). The analysis inferred the list of compound class (Table 2) present in the sample and the measurement of this energy will initiate the transition between the molecule's bond vibrational phases. The wide peak of absorption at $3200\text{--}3600\text{ cm}^{-1}$ is associated with stretching vibrations of O-H, indicating the presence of hydroxyl-containing compounds such as flavonoids, phenols, and carbohydrates²³. These compounds contribute significantly to the antioxidant properties of *Brassica oleracea*. The band at 3391.46 cm^{-1} is

Table 2: Compound class listed upon the wavenumber range in the infrared region.

Frequency range (cm^{-1})	Appearance	Functional Group	Class
3200-3600	Broad	O-H stretching	Hydroxyl groups (Polyphenols, Flavonoids)
3300-3500	Medium	N-H stretching	Amine groups (Proteins, Alkaloids)
3000-3100	Weak	=C-H stretching	Alkenes (Flavonoids, Terpenoids)
2850-2960	Strong	C-H stretching	Aliphatic Hydrocarbons
2100-2260	Medium	C≡C stretching	Alkynes
1600-1750	Strong	C=O stretching	Carbonyl groups (Aldehydes, Ketones, Esters)
1400-1600	Variable	C=C stretching (Aromatic)	Phenolic Compounds
1000-1300	Variable	C-O stretching	Alcohols, Esters, Carboxylic acids
600-900	Strong	C-H bending (Aromatic)	Aromatic rings

Table 3: GC-MS compounds listed based on peak area percentage value.

Retention time	Area (%)	Compound name	Molecular Formula
5.53	3.45	Hexadecane	$\text{C}_{16}\text{H}_{34}$
9.27	4.12	9-Octadecenamide (Oleamide)	$\text{C}_{18}\text{H}_{35}\text{NO}$
12.79	5.86	2,2,2-Trifluoroethyl 2-methyltetrahydro-5-oxo-3-furancarboxylate	$\text{C}_8\text{H}_9\text{F}_3\text{O}_4$
15.68	6.32	Phytol	$\text{C}_{20}\text{H}_{40}\text{O}$
18.92	7.25	Octadecanoic acid (Stearic acid)	$\text{C}_{18}\text{H}_{36}\text{O}_2$
20.91	8.50	Squalene	$\text{C}_{30}\text{H}_{50}$
23.14	4.75	Stigmasterol	$\text{C}_{29}\text{H}_{48}\text{O}$

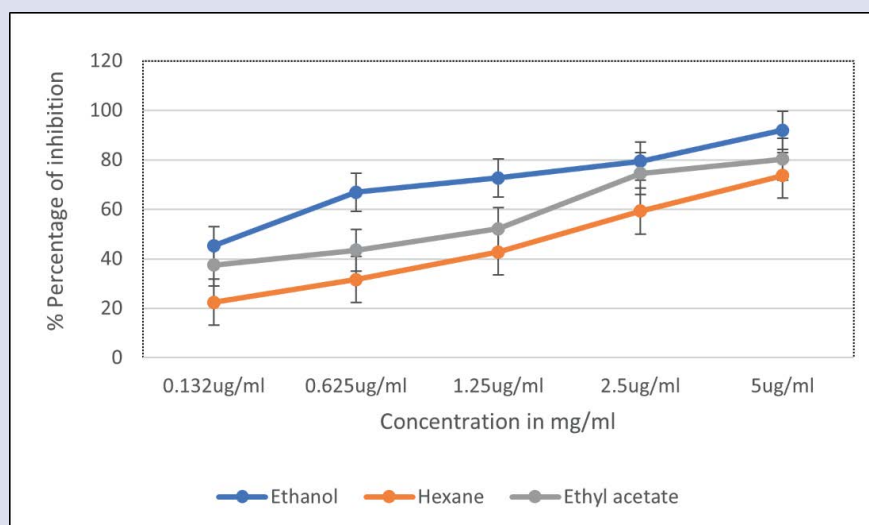


Figure 1: DPPH radical scavenging activity of BHE, BEAE and BEE.

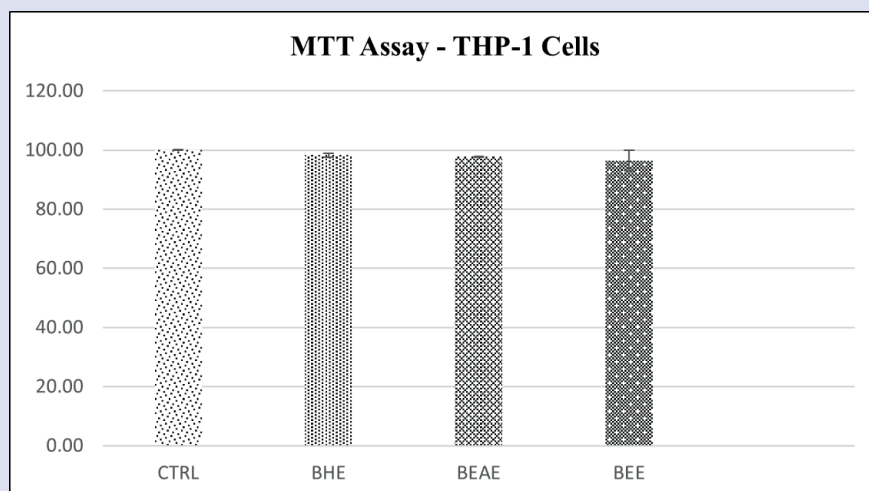


Figure 2: Anti-proliferative activity of hexane, ethyl acetate and ethanolic extracts of *Brassica oleracea* against THP-1 cell line at concentration 50µg/mL.

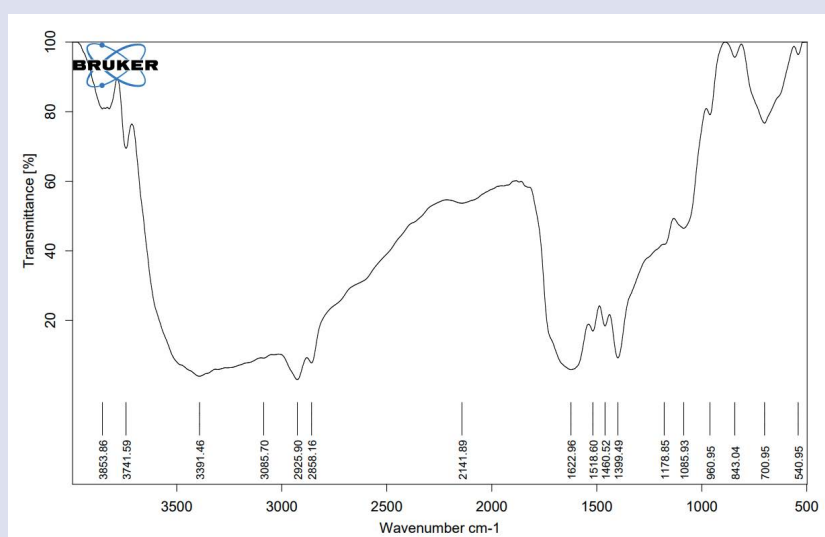
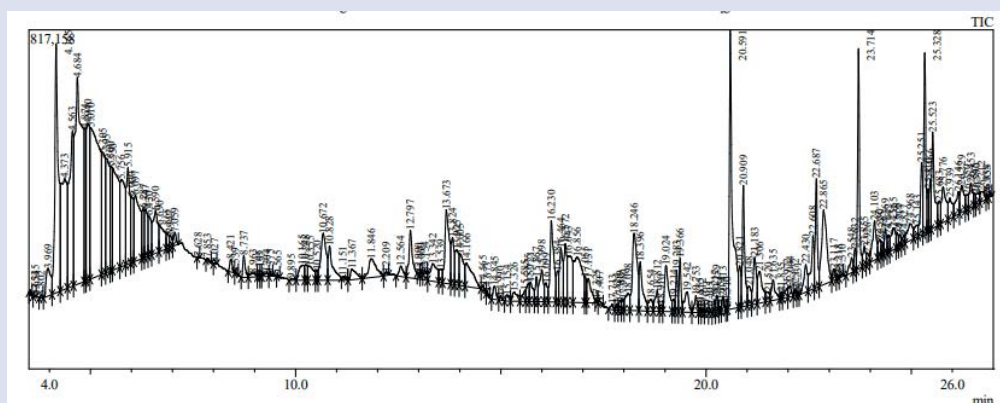


Figure 3: Determination of functional groups of ethanolic extract in the frequency range of 500 – 4000cm⁻¹.



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