

Extraction, Identification and Screening of *Brassica oleracea* var. *italica* Plenck (Broccoli) Floret to be an Alternative for Nanoparticle Formulations

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ABSTRACT

Background: Plant or plant extract act as a source of several abundant natural compounds such as flavonoids, phenolics, alkaloids, steroids, tannins, saponins and other nutritional compounds. The extract not only acts as reducing and stabilizing agents due to presences of various secondary metabolites for the bio reduction reaction but, also shows added pharmacological potential. Among others, a cruciferous vegetable like Broccoli is assumed to affect the growth of numerous forms of cancers since it contains multiple chemical constituents such as, Selenium, Sulphoraphene, Glucosinolate and Diindolylmethane which shows anticancer activity. **Objectives:** The study involves extraction of florets of *Brassica oleraceae* var. *italica* which is done by cold maceration process by using different solvents like water, ethanol, methanol and methanol: water (6:4) followed by phytochemical screening. **Methods:** Broccoli plant was collected from local farmer and Extraction of aerial part (Florets) was done by cold maceration by using various solvents such as water, Et: OH, Water: EtOH (6:4 ratio). The optimization of the extract with solvent selection was done by the observation of color, nature and also by the calculation of percentage yield, solubility concentration and phytochemical screening tests. Further, the optimized extract was subjected to calculate the total phenolic and flavonoids concentration. **Results:** The study involves collection of Broccoli from Local farmer. The plant was identified and authenticated as *Brassica oleraceae* var. *italica*. (Family: Brassicaceae) from Botanical Survey of India, Western Regional Centre, Pune by Ms. Priyanka A. Ingale, Scientist B (Voucher specimen No. RBC-3, BSI/WRC/IDEN. CER./2016/667). Extraction of florets of *Brassica oleraceae* var. *italica* which was done by cold maceration process by using different solvents likes water, ethanol, methanol and methanol: water (6:4) followed by phytochemical screening. **Conclusion:** The present study reflects that the extract of *Brassica oleraceae* var. *italica* Plenck shows major presence of phenolic and flavonoids as per phytochemical screening to be an alternative for the nanoparticle formulation.

Key words: Broccoli, Extraction, Identification, Screening, Qualitative and Quantitative Analysis.

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INTRODUCTION

Herbal medicine that forms an integral part of CAM has been testified to play a vital role in the management of breast cancer. Different medicinal plants including *Taxus baccata* (Pacific Yew), *Podophyllum peltatum* (Mayapple), *Camptotheca acuminata* (happy tree) and *Vinca rosea* (Periwinkle) have been

evaluated in clinical trials for breast cancer.^{1,2} Medicinal plants are a source of a large number of bioactive that are excellent anticancer agents as they have the efficacy to control the molecular mechanisms and various signaling pathways implicated in carcinogenesis such as inflammation,



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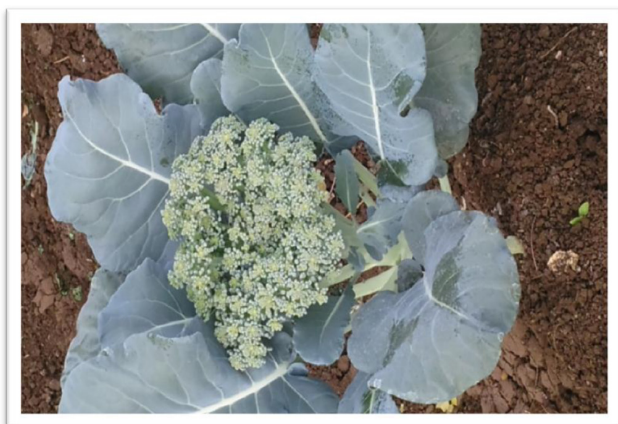


Figure 1: Broccoli Plant.

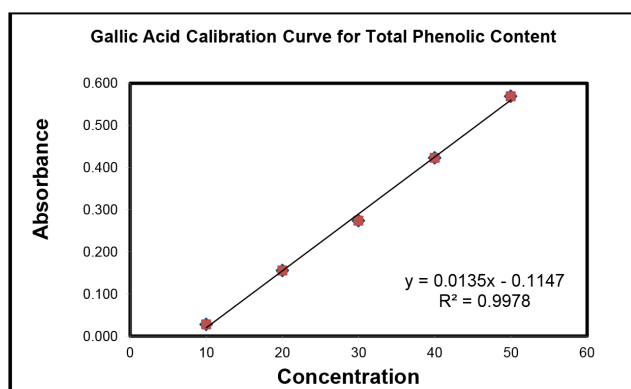


Figure 2: Gallic Acid Calibration Curve for Total Phenolic Content.

apoptosis, oxidation, cell cycle, cell proliferation, metastasis, invasion and angiogenesis.² Broccoli is a cruciferous vegetable which belongs to Brassica family. It is classified in the *Italica* cultivar group of the species *Brassica oleracea*. Broccoli (Figure 1), is considered as a good source of nutrients because it is rich in Vitamin C, Carotenoids, Fibre, Calcium and folate. Broccoli contains several compounds called isothiocyanates including selenium, sulforaphane and indole-3-carbinol, which possess anticancer activity. These constituents may act as cytotoxic and boost detoxifying enzymes in the body.³

MATERIALS AND METHODS

Selection and Procurement of Plant

Broccoli plant selected for study as it exhibited anticancer potential and used as capping and stabilizing agent to form and stabilized nanoparticles. The Broccoli was received as a gift from the local Farmer staying at Loni Kalbhor village near to Pune, Maharashtra, India.

Identification and authentication of Plant

The plant was identified and authenticated as *Brassica Cretica* Lam./ *Brassica oleracea* var. *italica* Plenck (Family: Brassicaceae) from Botanical Survey of India, Western Regional Centre by Ms. Priyanka A. Ingale, Scientist (Voucher specimen No. RBC-3, BSI/WRC/IDEN. CER/2016/667).

Drying and Pulverizing

The aerial parts Florets, Stalk, Roots and Leaves of Broccoli were obtained and dried in shade at a temperature not beyond 40°C. It crashed into a coarse powder with the help of mixer and allowed through the sieve number 16 and stored in a air tight and well-closed container at a moisture free place.⁴

Macroscopic study of plant material

The crude drugs observed for the presence of following macroscopically parameters i.e. Color, Odor, Taste and determination of external organic substance. The external organic substance such as insects, molds or other animal contaminations and parts of organ or organs from which the drug resulting other than the elements termed in the description and definition was determined by weighing 100 g of dried sample and spreading them over the white tile uniformly without overlapping, this sample inspected with the naked eye and the foreign matter was removed manually. Further, the weighed percentage of w/w was determined.^{5,6}

Extraction of Plant Material

Aqueous extract

The 1000 g of Broccoli Florets, Leaves, Stem and Root were broken separately into small pieces and macerated with water for seven days. After that, filtration was done to remove marc then pressing of marc done. Evaporation of solvent was done by employing rotary solvent evaporator. Finally, the obtained extract as weighed and its percentage in terms of the air-dried weight of the plant material were calculated.⁷

Ethanolic extract

The 1000 g of Broccoli Florets, Leaves, Stem and Root were broken separately into small pieces and macerated with ethanol for seven days. After that filtration was done to remove marc, then pressing of marc was done. Evaporation of solvent was completed by using rotary solvent evaporator. Finally, the extract gained with solvent was then weighed and its % in terms of solvent free weight of the plant material was determined.⁸

Ethanol and Water (6:4) extract

Broccoli (1000 gm of each) Florets, Leaves, Stem and Root, were broken separately into small pieces and macerated with a solvent concoction containing ethanol and water (6:4) for seven days. After that it was subjected to filtration to remove the marc and then pressing of marc done. Evaporation of solvent carried out by employing rotary solvent evaporator. Finally, the extract gained with solvent was then weighed and its % in terms of solvent free weight of the plant material was determined.^{9,10}

Observation and Inferences of Plant crude extracts

The prepared crude extracts was observed for the percentage yield, Color, Nature, pH etc.

Preliminary Phytochemical Qualitative Screening

The filtered extracts utilized as a test sample for primary qualitative identification of phytochemical constituents and the following phytochemical Tests was carrying out.¹¹⁻¹⁴

Tests for Carbohydrates

Molish's test (General test)

Two-three drops of α -naphthol and 1 mL of conc. H_2SO_4 was to 2 mL of test sample from the sides of the test tube to form two layers. The appearance of the violet color ring at the junction of two liquids in a test tube confirmed the presence of carbohydrates.

Test for Reducing Sugars

Fehling's Test

One mL each of Fehling's A and Fehling's B solutions were mixed thoroughly and boiled on water bath for 1 min. An equivalent quantity of test solution was further mixed to the test tube and boiled for 5 min. Development of a yellow precipitate, which turns brick red has observed, which shows the occurrence of reducing sugars.

Benedict's test

Test solution (1 mL) added into Benedict's reagent (2 mL); the solution was warmed and allowed to stand. Presence of sugars was indicated by the formation of red precipitation.

Test for Monosaccharides

Barfoed's test

Barford's reagent (2 mL) was taken into 1 mL of the test sample and boiled on water bath for two min and allowed to stand. Presence of sugar was indicated by red precipitation.

Test for Pentose Sugars

An equivalent quantity of test sample and HCl were added together and allowed for heating, phloroglucinol crystal was further added. Formation of red color, confirms the presence of pentose sugar.

Test for hexose Sugar

Selewinoff's test (for fructose)

Selewinoff's reagent (3 mL) and 1 mL test sample was mixed and heating was given on a water bath for 1-2 min. The red color was seen, which indicates the presence of hexose sugar.

Tollen's Phloroglucinol test (for galactose): Test solution (2 mL) added into a mixture of 2.5 mL conc. Hydrochloric acid and 4 mL phloroglucinol (0.5 %). Then it was heated. The reddish-yellow color appeared which indicates the presence of galactose.

Cobalt chloride test

Mixture of 3 mL test sample and 2 mL cobalt chloride was allowed for heating to boil and then it was kept for cooling. Few drops of Sodium hydroxide solution added. The solution looked greenish-blue due to presence of Glucose or purple indicated the identification of fructose or upper layer greenish-blue and lower layer purple confirms the presence of glucose and fructose.

Test for non-reducing Polysaccharide (Starch)

Iodine test

Test sample (3 mL) and few drops of dilute iodine solution were mixed together. The blue color give the impression if presence of starch which disappeared on heating and reappeared on cooling.

Test for Mucilage

A powdered drug material exhibited a red color after addition of ruthenium red.

Tests for Proteins

Biuret test

Two mL of NaOH (4 %) and a few drops of $CuSO_4$ (1 %) was mixed together in a 3 mL test sample. Proteins presence confirmed by observation of violet or pink color.

Million's test Million's reagent (5 mL) added into 3 mL of the test sample, White precipitation appeared which turned brick red on warming.

Tests for Amino Acids

Ninhydrin test

Mixture of 3 mL test sample and three drops of Ninhydrin solution (5%) heated in boiling water bath

for almost 10 min. Purple or bluish color appeared specifies the amino acids existence.

Tests for Fats and Oils

Filter paper test

Oils causes permanent stain to Filter paper.

Few drops of CuSO_4 and NaOH solutions added in ethanolic solution. The clear blue solution observed.

Tests for Glycosides

Test for Cardiac glycoside

Keller Killani test

Glacial acetic acid, one drop of FeCl_3 (5 %) and few drops of conc. H_2SO_4 mixed into 2 mL of extract, Identification of cardiac glycoside illustrated by the occurrence of reddish-brown color at the intersection of the two liquid layers and upper layer appeared bluish-green.

Legal's test

Pyridine (1 mL) and sodium nitroprusside (1 mL) was mixed into extract. Existence of cardiac glycosides was indicated by the formation of pink to red color.

Test for Anthraquinone glycosides

Borntrager's test

Three mL of extract was added with H_2SO_4 (Dilute). The resulting solution was subjected to boiling and then filtered. Equal amount of chloroform was mixed. This obtained solution was shaken well and processed for the organic solvent separation by addition of ammonia. The resulted ammonical layer changed to red from pink, which shows the existence of anthraquinone glycosides.

Tests for Saponin glycosides

Foam test

Dry powder of extract shaken robustly with water. The foam was observed.

Hemolytic test

Dry powder was added into few drops of blood located on a glass slide. Hemolytic region was appeared.

Test for Cyanogenic glycosides

Grignard reagent or sodium picrate test

Filter paper strip firstly was soaked in picric acid (10 %), after that it was soaked in sodium carbonate (10 %) and dried. Separately moistened powdered drug placed

Corked in a conical flask. Positioned the above filter paper on it. Presence of cyanogenic glycosides was confirmed on change in brick red or maroon color of filter paper.

Test for Coumarin glycosides

Small quantity of soaked sample was positioned in a test tube which was covered with filter paper pre moistened with dilute NaOH solution. The test tube was exposed to boil 15 min. After that the paper was detached and allowed to expose to light of UV. Presence of coumarins was designated by yellow fluorescence.

Test for Flavonoids

Shinoda test: Three drops of HCl, 5 mL of ethanol (95%) and magnesium turnings (0.5 g)

Test for Alkaloids

The extract evaporated. In the residue, HCl (Dilute) was poured; Resulting mixture was thoroughly shaken and was allowed for filtration. With filtrate subsequent identifications were executed.

Mayer's test

Mayer's reagent (few drops) was mixed into few mL of resulting filtrate. The Occurrence of alkaloid was designated by the development of precipitate.

Hager's test

Few drops of Hager's reagent were put into few mL of resulting filtrate. The incidence of alkaloids was confirmed by the establishment of yellow precipitate.

Wagner's test

Wagner's reagent (few drops) was mixed with few mL of filtrate. Incidence of alkaloids was specified by the formation of a reddish-brown precipitate.

Murexide test

Conc. HNO_3 (3-4 drops) added into 3-4 mL of the test solution, it was allowed to evaporate till dryness. In resulted cooled solution, NH_4OH (two drops) were added. Existence of alkaloids was indicated by the formation of purple color.

Test for Terpenoids

Knoller's test

Extract (5 mg) treated with 2 mL of 0.1% anhydrous stannic chloride in pure thionyl chloride observed a deep purple color which changes to red, indicating the presence of terpenoids.

Test for Steroids

Salkowski reaction

Two mL of conc. H_2SO_4 and chloroform was mixed into 2 mL of extract and shaken. Chloroform layer observed as red, whereas, greenish-yellow fluorescence was exhibited by the acid layer.

Test for Saponins

Foam test

Distilled water added to Solution of extract (1 mL) to make volume up to 20 mL and allowed shaking in a graduated cylinder for almost 15 min. Development of stable foam suggested the presence of saponins.

Extract (1 mL) treated with 1% lead acetate solution. Development of white precipitates indicated the incidence of saponins.

Test for Tannins and Phenolic Compounds

To 2-3 mL of aqueous extract, following given reagents (few drops) was added.

$FeCl_3$ 5 % solution: showed deep blue-black color.

Lead Acetate Solution: showed white precipitation.

Potassium Dichromate: showed red precipitation.

Bromine water: showed discoloration of bromine water.

Three drops each of saturated $FeSO_4$, H_2O_2 and NaOH solution added into 3 mL test sample. Presence of tartaric acid was indicated by the formation of a violet-colored solution.

Preliminary Phytochemical Quantitative Screening

Determination / Estimation of Total Phenolic Contents in the plant extracts

Total phenolic contents determined by Folin-Ciocalteu's reagent spectrophotometric method. The solution of methanolic extracts concentration of 1 mg/mL and 0.5 mg/mL used for the examination. Reaction concoction prepared by mixing of methanolic solution of extracts (0.5 mL), 10% Folin-Ciocalteu's reagent (2.5 mL) and 7.5 % $NaHCO_3$ (2.5 mL). Blank consisting of methanol (0.5 mL), 10 % Folin-Ciocalteu's reagent (2.5 mL) and 7.5 % of $NaHCO_3$ (2.5 mL). The samples after that incubated at 45°C for 45 min. The absorbance

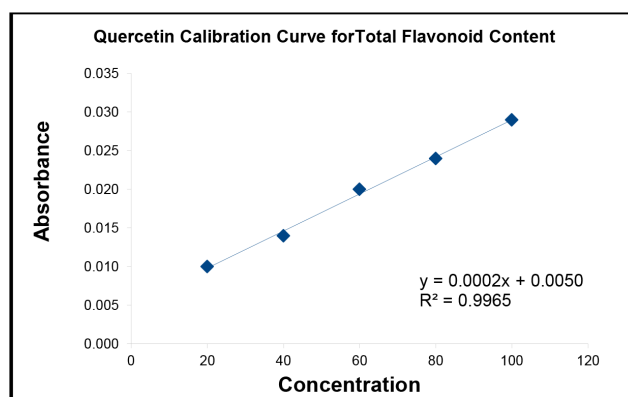


Figure 3: Quercetin Calibration Curve for Total Flavonoid Content.

was estimated using UV spectrophotometer at λ_{max} 765 nm. The same procedure implemented for gallic acid standard solution and the calibration line constructed. Depending on the observed absorbance, the concentration of phenolics was calculated (mg/mL) from the calibration line; then the content of phenolics in extracts was stated in terms of gallic acid equivalent.^{15,16}

Determination of Total Flavonoids Contents in the plant extracts

The total flavonoid content in extracts was estimated by the aluminum chloride colorimetric method. An ethanolic solution of the extracts, in the concentration of 1 mg/mL was used for the investigation. Extract (50 μ L) prepared up with methanol (1 mL), gently mixed with 4 mL of distilled water and then further with 5 % $NaNO_2$ solution (0.3 mL); In the resulting solution, after 5 min 10 % $AlCl_3$ solution (0.3 mL) was added, further the mixture was kept to stand for 6 min. Then, 1M NaOH (2 mL) solution was added and then the final volume of the taken to 10 mL with double-distilled water. The resulting concentration was allowed to keep aside for 15 min and absorbance was taken at 510 nm. The total flavonoids content was estimated from a calibration curve and the result was interpreted as mg quercetin equivalent per gram dry weight.^{17,18}

Result and Discussion

Selection, Procurement and Authentication of Plant

The Broccoli plant was received as a gift from the local Farmer Shri. Kaka Kalbhori staying at Loni Kalbhori village near to Pune. It exhibits anticancer potential. Plant authentication confirms the appropriate plant species used as materials for herbal formulation. The plant was identified and authenticated as Broccoli (*Brassica oleracea* var. *italica* Plenck) / *Brassica Cretica* Lam. (Family: Brassicaceae) from Botanical Survey of India,

Table 1: Macroscopic observation of *Brassica oleracea* var. *italica* Plenck Plant.

Sr. No.	Parameter	Observation
1	Color	Green
2	Odor	Characteristic
3	Taste	Characteristic
4	Determination of foreign organic matter	Nil

Table 2: Percentage Yield / Colour / Nature of Broccoli Floret Extract.

Sr.no	Part of plant	Solvent	Initial wt. of powder	Final wt. of powder	Wt. of Crude Extract	Crude Extract %	Colour of Extract	Consistency of Extract
1	Florets	EtOH	100 gm	89.13 gm	10.87 gm	10.87 %	Greenish	Semi-solid
		Water	100 gm	87.81 gm	12.19 gm	12.19 %	Greenish	Semi-solid
		Water: EtOH (6:4)	100 gm	86 gm	14.00gm	14.00 %	Brownish black	Semi-solid

Western Regional Centre by Ms. Priyanka A. Ingale, Scientist (Voucher specimen No. RBC-3, BSI /WRC/ IDEN.CER /2016/667).

Drying and Pulverizing

The aerial parts Florets of Broccoli were procured and dried below 40°C in a shade. It was crushed into a coarse powder with grinder and passed from the sieve number 16 and preserved in air tight well-closed container.

Macroscopic study of Plant Material

Macroscopic assessment of herbal material is the primary and prime footstep to confirm the identity and to examine the quality, purity and strength of the crude drug. The fresh Broccoli was observed for the presence of macroscopically parameters as shown in Table 1. The color was found to be green whereas odor and Taste were found to be characteristic. It was observed free from external organic substance which is in good agreement with reported observations.⁵

Extraction of Plant Material

Extraction of Plant part is a process that targets to extract definite constituents existing in plants. Extraction of aerial part of Broccoli (Florets) was done by cold maceration by using various solvents such as water, Et: OH, Water: EtOH (6:4 ratio). Depending upon the solubility of chemical constituents, the solvent Et:OH, Water: EtOH (6:4 ratio) was selected and optimized for further studies.^{5,9,10}

Screening of Plant Extract

% Yield / Colour / Nature

The crude extracts were observed for the presence of following parameters such as Percentage Yield / Colour / Nature. Table 2 indicates the % Yield / Colour / Nature of floret extracts by using various solvents. Highest % Yield was reported in hydroalcoholic solvent from 14 % for floret. The color of floret extract was reported brownish-black. The nature of floret extracts was found to be semi-solid.¹⁰

Table 3: Qualitative Analysis of Phytochemicals.

Tests /extracts	Ethanol	Water	Et: Water (6:4)
	F	F	F
Carbohydrate	-	+	-
Protein	+	+	+
Fats/ waxes	-	-	-
Glycoside	-	-	-
Flavonoids	+	+	+
Alkaloids	+	+	+
Terpenoids	-	-	-
Steroids	-	-	-
Saponin	-	+	+
Phenolics / Tannins	+/+	+/+	+/+

Whereas, F: Floret, +: Present and -: Absent

Table 4: Absorbance of Total Phenolic Content in Plant Extract.

Sr.No	Sample	Absorbance (nm)	Calculated concentration (µg/mL)
1	FE (1 mg/mL)	0.281	29.3 ± 2.354

Table 5: Absorbance of total Flavonoid Content in Plant Extract.

Sr. No.	Sample	Absorbance (nm)	Calculated concentration (µg/mL)
1	FE (1 mg/mL)	0.099	391.7 ± 16.493

pH of Extracts

The pH of floret extracts was observed as 3 which are in agreement with established values.

Phytochemical Study

Preliminary Phytochemical investigation: (Qualitative Analysis)

The crude extracts were observed for the existence of numerous phytoconstituents by carrying out different Phytochemical Tests.^{12,13} The sample containing Floret

extract of Broccoli has been chemically exposed to different tests to check the incidence of different chemical constituents like alkaloids, amino acids, carbohydrates, flavonoids, glycosides, tannins (phenolic compounds), starch and proteins and the results were shown in the Table 3, indicates the presence of required flavonoids, phenolics and alkaloids responsible for activity in optimized extract (Et: Water (6:4)), which was further evaluated for quantitative analysis.¹⁴

Preliminary Phytochemical investigation: (Quantitative Analysis)

Determination / Estimation of Total Phenolic Contents in the plant extracts

Phytochemicals are largely spread in plants with a capability to decrease the danger of various diseases. Phenolics are considered to be the wealthiest antioxidants in Human Cancers.¹⁵ The total phenolic content of the floret extract (FE) sample (1mg/mL) was determined from the calibration curve ($R^2 = 0.998$). It was found that $29.3 \pm 2.354 \mu\text{g/mL}$ (Table 4), which is equivalents/g to Gallic acid (Figure 2).

Determination of Total Flavonoids Contents in the plant extracts

Flavonoids are secondary plant metabolites accountable for the color and aroma of florets. In the course of disease (cancer), flavonoids obstruct with multiple signal transduction pathways and thus reduces proliferation and angiogenesis.¹⁷ The total flavonoid content of the Floret extract (FE) sample (1mg/mL), Table 5, was determined from the calibration curve ($R^2 = 0.9965$) and it was found that $391.7 \pm 16.493 \mu\text{g/mL}$ (Figure 3).

CONCLUSION

Herbal extracts of broccoli was prepared by cold maceration method using different solvents such as water, ethanol and water/ethanol. Further the phytochemical screening tests were carried out to identify the chemical constituents such as Alkaloids, Amino acid, Carbohydrate, Flavonoids, Glycosides, Phenolic compounds, Starch, Proteins present in the *Brassica oleracea* var. *italica* Plenck Floret extract. Through these studies, it was confirmed the presence of active phyto-constituents in optimized extract of water/ethanol, which are responsible for the capping and anticancer potential. The obtained extract was evaluated for quantitative analysis such as phenolic and flavonoid contents and it was found a significant quantity. The study proved that the *Brassica oleracea* var. *italica* Plenck

Floret extracts were capable of producing the pharmaceutical dosage forms i.e. green synthesis nanoparticles to treat disease (s).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

%; Percent; λ_{max} : Wavelength of maximum absorbance; °C: Degree celcius; **Cm**: centimeter; μg : Micro gram; **hr**: Hour; **Mg**: Milligram; **Min**: Minute; **mL**: Milliliter; μL : Microliter; r^2 : Regression coefficient; **RP-HPLC**: Reverse phase-high performance liquid Chromatography; **Rpm**: Revolutions per minute; **SD**: Standard deviation; t_R : Retention time; **UV**: Ultraviolet.

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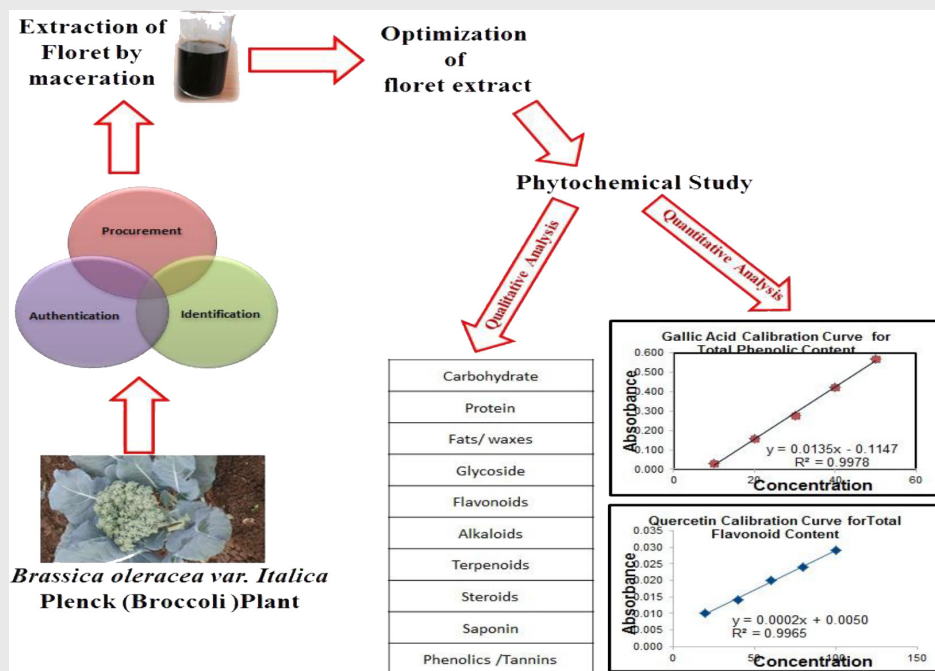
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SUMMARY

Medicinal plants are a source of a large number of bioactive that are excellent anticancer agents as they have the efficacy to control the molecular mechanisms and various signaling pathways implicated in carcinogenesis such as inflammation, apoptosis, oxidation, cell cycle, cell proliferation, metastasis, invasion and angiogenesis. Broccoli is a cruciferous vegetable which belongs to Brassica family. It is classified in the Italica cultivar group of the species *Brassica oleracea*. Broccoli, is considered as a good source of nutrients because it is rich in Vitamin C, Carotenoids, Fibre, Calcium and folate. Broccoli contains several compounds called isothiocyanates including selenium, sulforaphane and indole-3-carbinol, which possess anticancer activity. These constituents may act as cytotoxic and boost detoxifying enzymes in the body. Herbal extracts of broccoli was prepared by cold maceration method using different solvents such as water, ethanol and water/ethanol. Further the phytochemical screening tests were carried out to identify the chemical constituents such as Alkaloids, Amino acid, Carbohydrate, Flavonoids, Glycosides, Phenolic compounds, Starch, Proteins present in the *Brassica oleracea* var. *italica* Plenck Floret extract. Through these studies, it was confirmed the presence of active phytoconstituents in optimized extract of water/ethanol, which are responsible for the capping and significant anticancer potential.

PICTORIAL ABSTRACT



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