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DETERMINATION OF SECONDARY METABOLITES AND ANTIOXIDANT ACTIVITY OF *BRASSICA OLERACEA CAPITATA*

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ABSTRACT

To quantify the major secondary metabolites and the antioxidant potential of ethanolic extract of *Brassica oleracea*. The ethanolic leaves plant extract of *Brassica oleracea* was analaysed by HPLC and GC to determine various Phytochemicals. Free radicals scavenging activity of extract by using DPPH, NO and Super oxide radicals generated *in vitro*. The ethanolic extract of *B. oleracea* was found to contain alkaloids, terpenoids, phenols and flavonoids. The major flavonoid detected was quercetin and rutin. The *B. oleracea* was found to possess significant radical scavenging activity against DPPH, NO and superoxide anions the IC_{50} value of 52.0 µg/ml, 52.0µg/ml and 52.6µg/ml respectively and comparable to that of their corresponding IC_{50} value. The medicinal property of *B. oleracea* may be attributed to the presence of flavonoids and phenolic compounds with rich antioxidant potential. The therapeutic effect of this plant may be accounted for its counteracting action on free radicals *in vivo*.

KEY WARDS: *Brassica oleracea*, Phytochemicals, free radical, scavenging activity.

INTRODUCTION

Natural therapy for various human ailments purified with plant products has gained much attention now days, due to various side effects associated with allopathic medicine these can

be derived from any part of the plant like bark, leaves, stem, flowers, roots, seeds, etc., (Cragy and David, 2001). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farns Worth, 1989). Free radicals play an important role in various pathological conditions such as tissue injury, inflammation, neurodegenerative diseases, cancer and aging. The Compound that can scavenge free radicals has great potential in ameliorating these diseases (Coban *et al*., 2003). Inflammation is a disorder characterized by invasion of leucocytes and production of proinflammatory cytokines (Mantri and Witiak, 1994).

Broccoli is a form of cabbage, the *Brassica oleracea* capitata DC., or *Brassica oleracea* conica (H), of the mustard (Brassicaceae) family. It is a fast-growing, upright, branched, annual plant, 60-90 cm tall that is prized for its top crowns of tender, edible, green flower buds. Its thick, green stalks are edible too. It is native to Italy.

Broccoli and cauliflower are two derivatives of cabbage, both selected for their edible, immature flower heads. Broccoli is grown for the clustered green (or purple) flower buds that are picked before they open and eaten raw or cooked. The cauliflower head is a cluster of aborted, malformed flower buds that stopped developing in the bud stage. Cauliflowers come in white, lime green and purple varieties.

Broccoli has two different distinct forms. One is "sprouting broccoli," which makes a somewhat branching cluster of green flower buds atop a thick, green flower stalk, and smaller clusters that arise like "sprouts" from the stems. This form, called "calabrese" in Britain, is the most commonly grown form in the United States. The other type of broccoli makes a dense, white "curd" like that of cauliflower and is called "heading broccoli" or "cauliflower broccoli." This latter form is usually grouped with cauliflower, leaving the term "broccoli" restricted to sprouting varieties.

Like the other close relatives of cabbage, broccoli is native to the Mediterranean area and Asia Minor. It has been popular in Italy since the days of the Roman Empire. However, records indicate this vegetable was unknown in England until a relatively recent few hundred years ago. It has become popular in the United States only during this century.

It thrives in moderate to cool climates and is propagated by seeds, either sown directly in the field or in plant beds to produce transplants. Broccoli grows to about 0.75 m high, and

reaches harvest in 60 to 150 days, depending upon the variety and the weather. It is in flower from May to August, and the seeds ripen from July to September. The flowers are hermaphrodite (have both male and female organs) and are pollinated by bees. The plant can grow in semi-shade (light woodland) or no shade. It requires moist soil. The plant can tolerate maritime exposure.

Broccoli is available year-round but is a cool-weather vegetable that is best between January and March. Spring broccoli should be harvested in the early morning, because it wilts very rapidly in the sun. The broccoli head should be cut before the flower buds open. If the buds begin to open and the yellow flower petals begin to show, the head is over-mature and unfit for market. Cut the heads with a length of 23 to 25 cm from the base of the stem to the top of the head. The central heads vary from 6 to 12 cm in diameter. Light frosts do not hurt broccoli appreciably; therefore, harvest in the fall generally continues until the first freeze. Keeping this in view, the present study has been undertaken to investigate the phytoconstituents present in ethanolic leaf extracts of *Brassica oleracea*.

MATERIALS AND METHODS

Plant material and oil distillation: The medicinal plant of *Brassica oleracea* capitata DC leaves were collected from Chennai, Tamil Nadu, South India. The leaves were identified with the help of flora of presidency, Tamil Nadu and Karnatic flora (Gamble 1967; Matthew 1983) and standard references (Krtikar and Basu 1935).

Preparation of Extract: The dried and powdered leaves of *Brassica oleracea* (500 g) were extracted using soxhlet extractor by evaporating with 75% ethanol. The soxhlet extraction was carried out for 3 days and the extract was collected. The excess ethanol was evaporated by using vacuum evaporator. The sample is evaporated to dryness under boiling water bath at 55°C.

HPLC – UV analysis (Total Phenols): *Brassica oleracea* was subjected to solid phase extraction using column 5mm (4.6.mm) & peptides, small molecules were removed fractionation of neutral and acidic phenolic acids was also carried out simultaneously. The resulting fraction was then subjected to reverse phase high performance liquid chromatography (RP-HPLC). The total phenolics in *Brassica oleracea* was detected using, Stationary phase octadecylsil. Silica and mobile phase (A phosphoric acid: water (0.5: 99.5v/v) B acetonitrile). The UV detector was set at 220 nm with the flow rate adjusted to

1.0ms / min. The major peaks were identified and the retention times were compared with these of standards.

Fractionation of total Alkaloids: *Brassica oleracea* was detected using monobasic Phosphate as mobile phase (270ml. of Acetonitril). The liquid Chromatography is equipped with 235 nm detector & 4.6nm x 150 mm column. The flow rate was adjusted to 1.8ml / minute the major peaks were identified and the total alkaloids concentration were determined.

Fractionation of total Flavonoids: HPLC Chromatography (System Name: LACKROM L-7000 MERCK, Proc Method – HITECHI) total flavonoids. The total flavonoids in the extract was determined by using octadecysil silica gel as stationary phase and acetonitril, sodium dihydrogen phosphate with dilute orthophosphoric acid as mobile phase. UV detector was set at 350nm with flow rate of 0.5ml/min. The major peaks in *Brassica oleracea* were determined in comparison to the retention time of standards run at identical conditions.

Gas chromatography (GC analysis of terpenoids): The terpenoids level was measured GC using capillary column coated with macrogol 20000R and nitrogen as carrier gas. The flame ionization detector was set at the flow rate of 0.4ml/min & used as standard.

Free radical scavenging activity

Diphenyl – 2- Picrylhydrazyl (DPPH) radical scavenging activity:

DPPH radical scavenging assay is a commonly recommended method for assessment of antioxidant potential of plant extracts. The assay is based on the ability of DPPH, a free radical which get decolorized in the presence of antioxidants. To 200ml of ethanolic solution of DPPH (1µg/ml) various concentration of (20mg –100 µg/ml) in water were added and incubated at 370C for 30 min in dark and the absorbance was measured at 517nm. Ascorbic acid was used as the reference standard. The percentage scavenging of DPPH free radical was calculated and compared with that of the standard ascorbic acid. The IC_{50} value also determined.

Superoxide anion scavenging activity (Nishkimi *et al*., 1972)

The method was applied for the measurement of *Brassica oleracea* superoxide anion scavenging activity, Briefly 312µm Nitroblue tetarzolium in 120 µm phosphate buffer 74 were added to an aliquots of *B. oleracea* (20-100µg/ml) the reaction was started by adding 100ml of phenazinemethosulphate (120mm prepared in phosphate buffer pH 7.4) and the

colour change was monitored at 560nm against water blank querceutin was used as the positive control.

Nitric oxide scavenging activity

The nitric oxide scavenging activity of the aqueous extract was measured by taking various concentrations of *Brassica oleracea* and standard. Ascorbic acid (20-100µg/ml) dissolved in phosphate buffer (0.025m, pH 7.4) and incubated with sodium nitroprusside (5mm) in standard phosphate buffer at 25^0C for 5 hrs. After the incubation, 0.5ml of the reaction mixture was added with 0.5ml of Griess reagent (equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% napthlthyl ethylene diamine dihydrochloride in water). The absorbance of the chromophore formed was read at 540nm. The activity was compared with that of similar concentration of Ascorbic acid (Sreejayan and Rao, 1997).

RESULT AND DISCUSSION

Preliminary phytochemical screening of ethanol extract of *Brassica oleracea* revealed the presence of alkaloids, flavonoids, terpenoids and phenolic compounds which are essential to prevent diseases and to maintain a state of well being. Recent studies have been focused on finding the natural substance of medicinal plant that decrease the inflammation and reduce oxidative stress and there by counteracting the macromolecular damage. It is well known that reactive oxygen species interact with key bimolecular such as proteins and enzymes which regulate major metabolic path way and decrease their functional efficiency.

Table – 1: Quantiative Phytochemical Analysis

S. No.	Phytochemicals	Quantity mg/gm of dry material
1.	Alkaloids	1.45
2.	Terpenoids	0.88
3.	Total phenols	5.27
4.	Gallic acid	4.34
5.	Cinnamic acid	0.41
6.	Coumaric acid	0.32
7.	Flavonoids	1.07
8.	Rutin	0.224
9.	Quercetin	0.662

Shows that *Brassica oleracea* contains (Sheety and Wahiqvist, 2004) rich amount of bioactive compounds which exhibit antioxidant property the quantitative analysis revealed that *Brassica oleracea* contain rich amount of phenolic compounds and flavonoids. It is well

known that plant flavonoids and phenols in general are highly effective in scavenging free radical and providing antioxidant defense in living cells. Ployphenols and flavonoids isolated from plants medicinal have been used for the prevention and cure of various diseases which are mainly associated with free radicals.

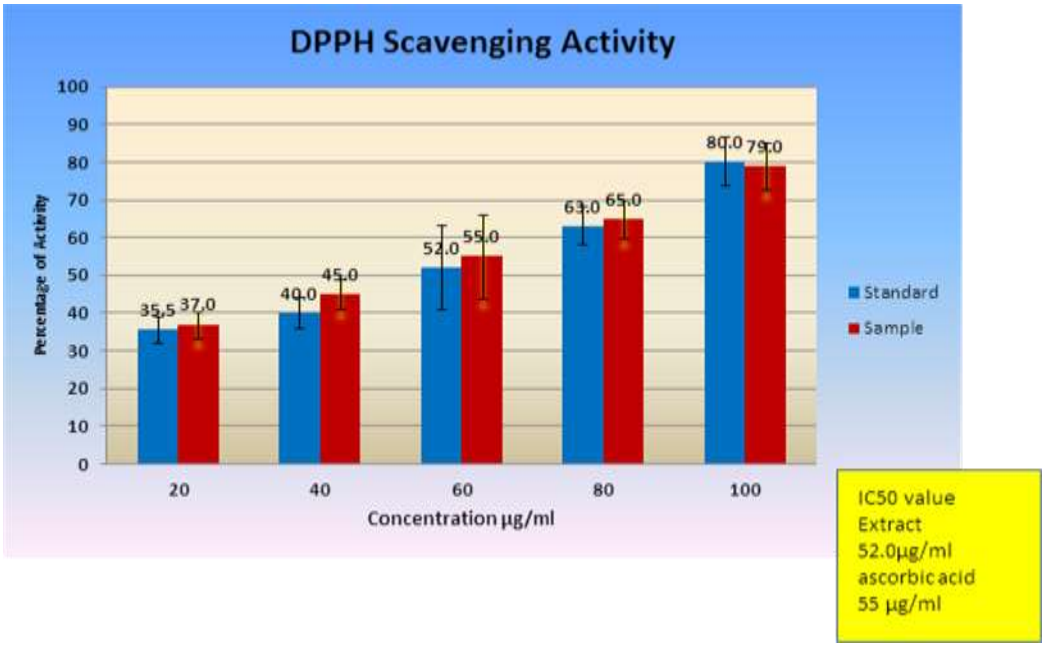
HPLC analysis reveals that the extract was found to be rich in Alkaloids (1.45 mg/g) terpenoids (0.88mg/g) and phenols phenolcs (5.27 mg/g). *Brassica oleracea* also contain flavonoids such as Rutin (0.224mg/g) and quercetin (0.662 mg/g) many reports demonstrate that antioxidant principle present in medicinal plants are responsible for their therapeutic potential (Larson, 1988). The flavonoic compound such as quercetin and Rutin are formed to be responsible for anti-inflammatory and anticancer properties proliferate by their terminating action of free radicals (Shahidi and Wana Sundara, 1992). Alkaloids have many pharmacological activities including anti cancer& anti arthythmic effect (Cordell, 1983). Alkaloids are known to reduce the inflammation level significantly. This result shows that *Brassica oleracea* containing which could be accounted for the antioxidant and anti-inflammatory effects.

It may lead to oxidative stress. The Natural phytonutrients presents in fruits and vegetables scavenge the free radicals and protect the cells from oxidative damages. The phytonutrients present in *B.oleracea* the responsible for the traditional claim by the test drug.

Reactive oxygen species and free radicals known as super oxide anions, hydroxy radicals, hydrogen peroxide are the major class of highly reactive species derived from normal all metabolism of major nutrients (Nadiu *et al*., 2014). These highly reactive free radicals if not counteracted and inactivated by cellular antioxidants. The DPPH is decolourised nature it receives electron or hydrogen atom from antioxidants and the extend of decolorisation represents the antioxidant potential of the test compounds. The result obtained in these investigation shows that *Brassica oleracea* possess a potent scavenging activity against DPPH radicals. The scavenging activity was comparable to that of standard ascorbic acid.

The IC_{50} value of *Brassica oleracea* (52.0µg/ml) was found to be nearer to that of standard ascorbic acid (55µg/ml) super oxide anion scavenging activity.

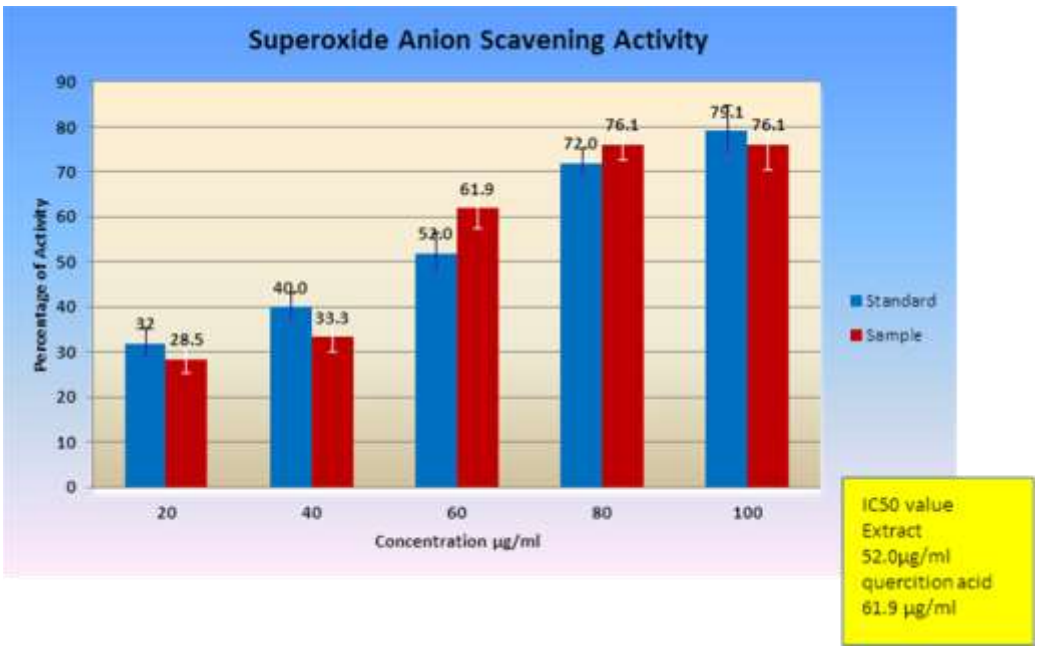
Calculate the percentage inhibition = (optical density of Control- Optical density of Test / Optical density of Control) x 100



DPPH Scavenging Activity of *Brassica oleracea* and Standard ascorbic Acid

Super oxide anion scavenging activity of *Brassica oleracea* was found to possess comparable free radical scavenging activity against super oxide anions when compared to that of standard quercetin. The IC_{50} value was found to be (52.0 ug / ml and in 61.9ug/ml) for MIT respectively.

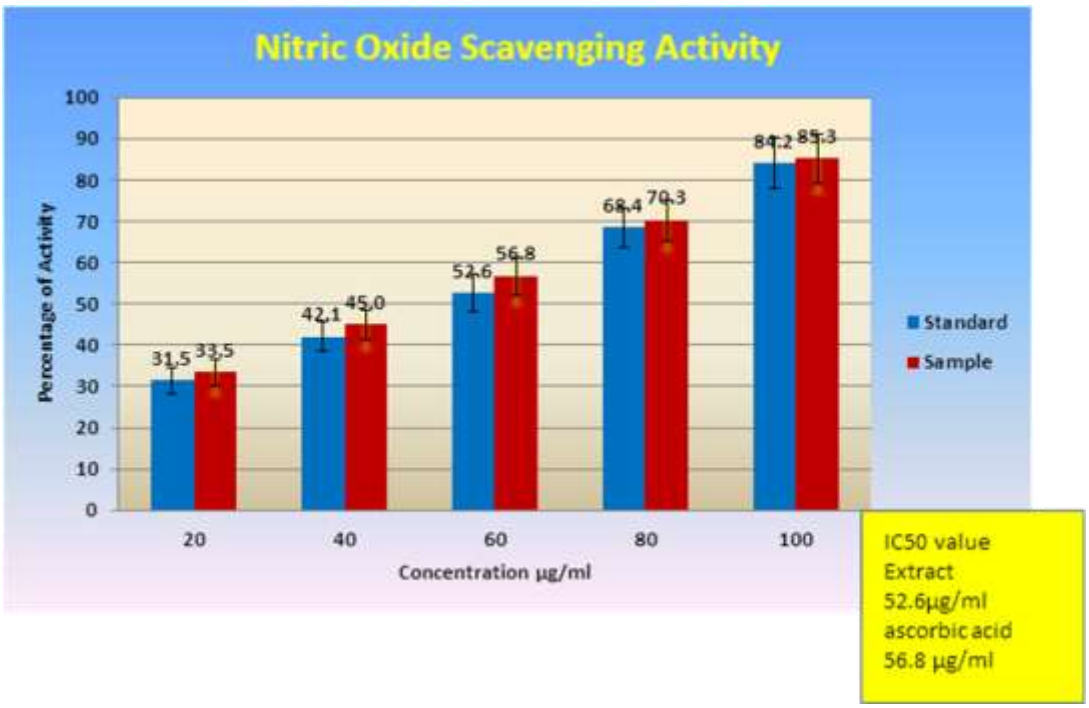
Calculate the percentage inhibition = (optical density of Control- Optical density of Test / Optical density of Control) x 100



Super oxide anion scavenging activity of *B. oleracea* and standard quercetin

The superoxide onions are toxic intermediates formed during inflammatory process and found to enhance the risk of inflammation related disorders such as arthritis and atherosclerosis. Super oxide anion is a free radio that plays an important role in the formation of reactive oxygen species such as hydrogen periode, hydroxyl / radicals, or singlet oxygen in living organism. Reported that the therapeutic activity of medicinal plants can be determined by superoxide activity were reported by Korycka and Richardson (1978).

Nitric Oxide scavenging activity is on important chemical mediator generated by endothelial cells, macrophages, neuron & it is involved in the regulation of various physiological process like control of arthritis, cytotoxice effects Alzhemer's disease (Sainani *et al*., 1997). No formation is toxic to living organism and it was found that *B. oleracea* significantly scavenges the nitric oxide and the effect was comparable to that of standard Ascorbic acid at similar concentration with IC_{50} value (52.6µg/ml and 56.8 µg/ml) of and respectively.



Nitric oxide scavenging activity of *B. oleracea* and standard Ascorbic acid.

Calculate the percentage inhibition = (optical density of Control- Optical density of Test / Optical density of Control) x 100

The result of preliminary phytochemical screening shows the presence of flavonoids such as quercetin and rutin, phenolic compounds and Alkaloids in the Plant. A large number of these compounds are known to possess strong antioxidant properties. The free radical scavenging

activity of *Brassica oleracea* revealed that they can be used for the prevention or treatment of human diseases such as cancer, arthritis, diabetes mellitus which are associated with oxidative stress.

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