Original Research Article

A Study on Antioxidant and Antimicrobial activity of Organic and Inorganic Cauliflower Greens Powder (*Brassica oleracea var. botrytis* L)

Abstract

Cauliflower greens is one such popularly consumed vegetable, possessing potent bioactive components where the leaves of the vegetables are often neglected or discarded and used as fodder. Hence an attempt was made to analyze the presence of different antioxidants such as DPPH, FRAP, SOD and Total antioxidant contents there by exploring the potential benefits of these leaves which is solely ignored by majority of population. Cauliflower have a very high waste index, and this result is throws a light on the importance and the properties of cauliflower leaf, thereby reduce the food wastage.

***Key Word*:** DPPH, Total antioxidant, SOD, FRAP, Antimicrobial activity

1. INTRODUCTION

Cauliflower is one of several vegetables in the species *Brassica oleracea*, in the family *Brassica oleracea var. botrytis* L. It is an annual plant that reproduces by seed. Typically, only the head (the white curd) is eaten. The cauliflower head is composed of a white in florescence meristem. Cauliflower heads resemble those in broccoli, which differs in having flower buds. Brassica oleracea also includes broccoli, brussels sprouts, cabbage, collard greens, and kale, though they are of different cultivar groups[1].

Cauliflower contains several phytochemicals which are beneficial to human health. It contains sulforaphane which protect against cancer, glucosinolates, carotenoids, indole-3-carbinol, isothiocyanates, dithiolethiones and phenols that enhances DNA repair and acts as an estrogen antagonist, slowing the growth of cancer cells. A high intake of cauliflower has been associated with reduced risk of aggressive prostate cancer. The leaf juice of cauliflower was found to possess antibacterial activity. Since the literature regarding the present work is meager an attempt has International Journal of been made to evaluate the antimicrobial activity and phytochemical analysis of Brassica oleracea extracts against the pathogenic microbes.

Organic production is global concern since consumption of organic vegetables has high growth in the developed countries. However, growing awareness of organic production in terms of soil health, sustainable production and environmental hazards, and healthy food consumption is also quite appreciable in various places. Apart from this, some countries has tremendous opportunity of organic vegetable production for the increased farm income because of growing demand of organic vegetables to health-conscious elite consumers in the country and export to its neighbor international markets[2].

Vegetables have been analyzed as potent medicine and man is able to obtain from them a wondrous assortment of industrial chemicals. In recent years population continues to explode and microbial disaster may occur. So vegetables with possible antimicrobial activity should be tested against an appropriate microbial model to confirm its activity and to ascertain the parameter associated with it. Cruciferous vegetables are one of the dominant food crops which have high vitamin C, soluble fibre and contain multiple nutrients and photochemical with potential anticancer properties. *Brassica oleracea* (Cauliflower) belongs to the family Brassicaceae is an annual plant that reproduces by seed. Cauliflower is low in fat, but high in dietary fibre, potassium, folate, water and vitamin and possesses a high nutritional density [3, 4].

2. MATERIALS AND METHODS

2.1 Collection of sample

The organic cauliflower greens was collected from the local farm area of Ooty, The Niligiris District Tamilnadu, India and the Non organic leaf was collected from the local Vegetable shop of Coimbatore Tamilnadu India.

After the collection, the leaves were dried under shade of sunlight (for 3-5days). Hence the plant material is a very complex matrix composed of different variety of organic compounds, and which may sensitive to the direct sunlight and heat. Further dried samples were stored in air tight container for further study.

2.2 Extract preparation

10% of extract was prepared by dissolving 10 gm in 100ml of the distilled water. This extract was incubated at 40 ^{0}C for 24hrs in the rpm of 60 – 70 in an orbital shaker. After incubation the extract was filtered through Whatman No.1 filter paper and used for further study.

2.3 Analysis of Antioxidant

2.3.1 DPPH

Free radical scavenging activity of the extracts was determined by DPPH assay. DPPH solution (0.004%, w/v) was prepared in methanol. Stock solution (1 mg/ml) standard ascorbic acid (0.05 g/ml) were prepared using methanol. 200, 400, 600, 800, 1000µl of the sample solution and 1ml of DPPH solution was added along with 0.4 ml of 50mM TrisHcl buffer, tube was incubated in dark for 30 minutes and the reading was measured at 517nm using spectrophotometer (LT 291 Labtronics microprocessor). Methanol was used as a blank, and the mg/g of the DPPH was calculated by using the ascorbic acid as a standard [5].

2.3.2 FRAP

Ferric reducing capacity of the extracts was investigated by using the potassium ferricyanide-ferric chloride method. 1ml of the extracts was mixed with 0.1M phosphate buffer solution and 2ml of the 0.1% potassium ferricyanide. The mixture was incubated at 50^0C for 30minutes and 2 ml of the 10% trichloro acetic acid solution was added to stop the reaction, by centrifugation at 5000rpm for 5 minutes the clear solution was separated and added 0.1% $FeCl_3$ solution (2ml) this was measured at 700nm using spectrophotometer (LT 291 Labtronics microprocessor). Mg/g of the FRAP was calculated in mg/g of ascorbic acid.

2.3.3 Total antioxidant activity

The total antioxidant activity of the sample was determined by using the phosphor molybdenum method. The assay was based on the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of green phosphate complex at acid pH. 0.2 ml sample was combined with 2 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The solution was incubated at 90°C for 90 mins. After cooling in room temperature, the absorbance of the solution was measured at 695 nm using a spectrophotometer against the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid with methanol [6] .

2.3.4 SOD

Superoxide dismutase (SOD) activity was determined by the inhibition in photoreduction of nitrobluetetrazolium by the SOD enzyme. To the 0.1ml of sample added 1ml of the reaction mixture I (1ml of 50mM PBS, 0.075ml of 20mM L Methionine, 0.04ml of 10mM hydroxyl amine hydrochloride, and 0.1ml of 50mMEDTA) and incubate the sample at 30^0C for 5minutes. After incubation added 50µM riboflavin and the sample was allowed to expose under 200W fluorescent light. 1ml of the reaction mixture II (1% Sulphanilamide in 5% phosphoric acid) was added and the measurement was read at 543nm under spectrophotometer [7].

$$\% \text{ inhibition of nitrite formation} = 1 - \frac{AS}{AC} \text{ X } 100$$

2.4 Antimicrobial activity [8]

2.4.1 Antibacterial activity

Antibacterial activity of the sample was identified by using well diffusion method against the bacteria. Mueller hinton agar (39gm in 1000ml) was prepared and swabbed 70µl of the bacterial culture (*E.coli, S.aureus, P.aeruginosa, K.pneumoniae, B.subtilis*) using cotton swab and well were made with corck borer followed by the sample (cauliflower greens) was added (50µl). Antibiotic disc (cefazolin 30mcg) was placed as a positive control, distilled water was used as negative control, the plate was incubated 37^0C for 24 hrs. After incubation anti bacterial activity of the sample was confirmed based on the zone of inhibition in mm.

2.4.2 Antifungal activity

For the antifungal study malt agar was prepared (39gm in 1000ml of distilled water, followed by autoclaving) and poured to petri plate. After solidification 80 µl fungal culture of *A.niger, A.flavus* and *A.terreus* was added and spread, using above said method sample also added and incubated 3-5 days at 30^0C. 5 µl of Fluconazole was used as a standard (10mg/ml), After incubation zone of inhibition was measured in mm.

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity

3.1.1 DPPH

Much research confirms that plants and vegetative source of foods are rich in antioxidants, which play an essential role in the prevention of free radical related diseases [9]. Antioxidants are the compounds and the free radicals mediated oxidative process are intervening with initiation, propagation and termination [10]. The increasing concentration of the cauliflower leaves were showing increasing DPPH activity and compare with inorganic sample organically cultivated sample shows higher activity, the result is presented in Table 1.

Table 1; DPPH radical scavenging capacity

Sample in different concentration (µl)	**DPPH in mg/g**	
	In organic	**Organic**
200	28.5	31.4
400	31.3	34.5

600	34.5	37.1
800	36.8	39.6
1000	41.0	43.4

DPPH radical scavenging activity of organic and inorganic cauliflower greens powder were determined and expressed in milligram. The DPPH radical is stable due to the delocalization of a spare electron over the molecule, thus preventing dimer formation. This radical is used in the DPPH radical scavenging capacity assay to quantify the ability of antioxidants to quench the DPPH radical. In another study [11] studied the bioactive compounds and antioxidant activity of the cauliflower and processed cauliflower and reported that the processed white cauliflower extract having the radical scavenging capacity from 35.13 % to 68.91%.

3.1.2 FRAP

The assay was based on the reducing power of a antioxidant. A potential antioxidant will reduce the ferric ion (Fe3+) to the ferrous ion (Fe2+); the latter forms a blue complex (Fe2+/TPTZ), which increases the absorption at 700nm. Among the two samples, Inorganic cauliflower greens shows 21mg/g and organic cauliflower green extract shows 23mg/g of FRAP content. These comparative results reveal that the organic extracts have higher antioxidant content.

[12] Reported that the FRAP assay has also been used as part of a quality control system in the agri-food industry, to measure the effect of genetic variation, storage, growing conditions, and season on the "total antioxidant content" of foods. Total antioxidant content of blueberries of the same cultivar grown in the same field can vary by up to 25% depending on the harvesting year, and variation of up to 47% in total antioxidant content is seen in different cultivars grown in the same area and harvested in the same year.

3.1.3 Total antioxidant activity

Total antioxidant capacity (TAC) is the measure of the amount of free radicals scavenged by a test solution, being used to evaluate the antioxidant capacity of biological samples. In the present study the total antioxidant capacity of the organic and inorganic cauliflower was measured spectrophotometrically at 695nm and the mg/g of the total antioxidant was found with the standard ascorbic acid. Organic and inorganic cauliflower greens shows 640mg/g and 741mg/g of the total antioxidant activity respectively.

The evaluation of the total antioxidant capacity (TAC) may bean appropriate tool to determine the additive antioxidant properties of plant foods [13]. The importance of TAC as a

novel instrument to estimate the relationship between diet and oxidative stress-induced diseases, is presented in recent studies [14].

3.1.4 SOD

To screen the SOD activity the effect of the cauliflower extracts on enzyme activity was measured spectrophotometrically and the percentage of inhibition was calculated. The ability to reduce NBT by PMS-NADH coupling can measure the superoxide radicals generated from dissolved oxygen. Superoxide free radicals showed maximum inhibition in organic form of cauliflower greens compare with inorganic form of cauliflower greens. Inorganic cauliflower shows 58.65% and organic cauliflower green shows 64.12% of nitrate inhibition.

A unit of enzyme activity is generally defined as the amount of enzyme that inhibits the reaction of O_2 − with an indicator by 50%. These are indirect methods involve the inhibition by SOD on a product resulting from the reaction between an indicator and O_2 . The O_2 – is produced enzymatically or non-enzymatically during the autoxidation of a compound [15].

3.2 Antimicrobial activity

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Here well diffusion method was used to evaluate the antibacterial and antifungal activity of the organic and inorganic cauliflower greens. The result is presented in Table 2.

Table 2 ; Antimicrobial activity

Microorganism used	**Zone of inhibition in mm**			
	Organic	**Non-organic**	**Water**	**Disc**
E.coli	5mm	2mm	Nil	7mm
S.aureus	5mm	Nil	Nil	Nil
P.aeruginosa	4mm	Nil	Nil	1mm
K.pneumoniae	Nil	Nil	Nil	Nil
B.subtilis	6mm	4mm	Nil	5mm
A.niger	Nil	Nil	Nil	4
A.flavus	Nil	Nil	Nil	Nil
A.terreus	Nil	Nil	Nil	Nil

The antimicrobial activity shows that organic cauliflower greens have higher zone of inhibition compare with the inorganic cauliflower sample. In the present study none of the extract shows any antifungal activity and both extract shows antibacterial activity except *K.pneumoniae*. The results are coinciding with the study of [16] highlighted that the tested extracts of *Brassica oleraceae* var. *botrytis* showed potential antibacterial activity against *Bacillus sp, Escherichia coli, Staphylococcus aureus, Klebsiella* sp, and *Pseudomonas* sp. Antibacterial activity of acetonic extracts of *Brassica oleraceae* exhibited highest zone of inhibition 29mm in diameter, against *S.aureus*. The zone of inhibition against *Bacillus sp, Escherichia coli, Klebsiella* sp, *Pseudomonas* sp were 20 mm, 27 mm,22 mm and 26 mm respectively. The study suggested that bioactive substances from this plant can therefore be employed in the formulation of antimicrobial drugs for the treatment of various bacterial infections.

Prasad [17], carried out antimicrobial assay with different solvent extracts of cauliflower leaves against some pathogenic bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. The chloroform extract of cauliflower leaves showed highest zone of inhibition against *E.coli* with a zone of 34 mm.

Satya *et al*, [18], evaluated the in vitro antimicrobial activity of ethanol, methanol and aqueous extracts of vegetable leaves from *Brassica oleraceae* var. *botrytis* (Cauliflower) against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella paratyphi* and *Salmonella typhimurium*. The highest zone of inhibition exhibited by aqueous extract was 10 mm against *Staphylococcus aureus.*

4. CONCLUSION

The results of the study reveal that the presence of antioxidant contents of DPPH, FRAP, SOD and Total antioxidants are high in organically cultivated cauliflower extract compare with the inorganically cultivated cauliflower greens. Antioxidants have a positive effect on the health even though it is in smaller amounts, it can play a significant role in maintaining good health. Due to lack of knowledge the cauliflower leaves are not included in the daily diet, this study is an attempt to find the antioxidant and antimicrobial activity of the sample to disclose the medicinal importance and can be included as functional food.

5. REFERENCE

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