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QUANTIFICATION OF ANTIOXIDANTS IN UNDERUTILIZED VEGETABLE LEAVES

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ABSTRACT

Introduction: Green leafy vegetables are an excellent source of bioactive compounds beyond human nutrition. There are many underutilized vegetable leaves which are equally a power house of bioactive compounds with reference to antioxidants. Much literature confirms that brassica vegetables are high in antioxidants but the antioxidant studies these vegetable leaves are scarce as they are underutilized and neglected hence the present study was framed.

Aim: To analyze the total antioxidants, total phenols and total flavonoids in the leaves of cauliflower radish and beetroot leaves.

Methodology: Total antioxidants were analysed using Dpph method and phenols by Folin-Ciocalteu method and Total flavonoids were determined spectrophotometrically.

Results: The observations were read in triplicates. The data was analyzed statistically showing the presence of total antioxidants, total phenols and total flavonoids in leaves of cauliflower radish and beetroot which can a therapeutic role in the health of humans.

Conclusion: Hence the present study reveals the benefits of these underutilized leaves which are packed with antioxidants that can prevent from all degenerative diseases and these antioxidant leaves can be also treated in formulation of functional foods and in food and health industry.

Key Words: Underutilized vegetable leaves, Total antioxidants, Total phenols, Total flavonoids, Degenerative diseases

INTRODUCTION

Humans have used different types of leaves as food since time immemorial. Plants possess many phytochemicals with various bioactivities including, carotenoids, ascorbic acid, tocopherol and polyphenols¹. A vegetable includes leaves, stems, roots, flowers, seed, fruits, bulb and tubers². Among the food groups green leafy vegetables has a significant place due to its abundant presence of vitamins, minerals bioactive health promoting compounds such as antioxidants and phytochemicals for health promotion and also in supply of, which provides benefits beyond basic nutrition.

Though the availability of these leaves are cheap, they are highly perishable affected by post harvest losses, storage, handling and transport. Recently more attention is focused on importance of antioxidants for maintaining healthy life through many researches, books and media updates. Reactive Oxygen Species are a class of highly reactive molecules

formed during aerobic life.³ A good source of natural antioxidants with reference to phenols and flavonoids play a significant role in prevention against the formation of reactive oxygen species in maintaining a healthy body. Antioxidants are free-radical scavengers which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species⁴. The most commonly used synthetic antioxidants are Butylated Hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Propylgallate (PG) and butylated hydroquinone which have side effects such as liver damage and carcinogenesis⁵. There is an emerging need for the application of natural antioxidant having less or no side effects, for use in foods or medicinal preparation in order to replace synthetic antioxidants. There is a growing interest in natural additives as potential antioxidants⁶. Development of safer natural antioxidants from extracts of spices and other plant materials that can replace synthetic antioxidants is of interest⁷. In this respect there are many

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varieties of green leafy vegetables in our locality, which are discarded and not used properly for human consumption that is antioxidant rich. Brassicas are known to possess antioxidant activity⁷. Brassica vegetables such as cauliflower and broccoli are popular and are among the most consumed in the world. Many researchers have proven the presence of potential antioxidants in cauliflower on reviewing these perspectives an attempt was made to analyze the antioxidant activities in the leaves of cauliflower, beetroot and radish belonging to similar plant family having high waste index.

MATERIALS AND MEHODS

The leaves of vegetables that are easily available, suitable for consumption, free of cost but neglected, discarded and are used as fodder for animals were the criteria's considered in selection of leaves namely cauliflower beetroot and radish. The nutritional knowledge about these leaves is also ignorant and hence avoided by majority of human population. The selected leaves were purchased fresh from the farm in Ooty Tamil Nadu. The leaves were separated from the stalks and washed twice under running tap water and blanched for 10-15 sec. Later each variety of leaves was chopped and was spread on a filter paper at room temperature for two hours for the remaining water to drain the excess water from the leaves. It was then dried at 40°C over-night (12 hours) in hot air oven. The dried leaves of each variety were ground to a fine powder and packed in air tight containers for the analysis.

1. DETERMINATION OF TOTAL ANTIOXIDANTS

DPPH method is widely used to determine antiradical/ antioxidant activity of purified phenolic compounds as well as natural plant extracts⁸. Assays based on the use of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2'-azino-bis (3-ethyl-benzthiazoline-6- sulfonic acid) (ABTS) radicals are among the most commonly used spectrophotometric methods for determination of the antioxidant capacity of foods, beverages, plant extracts and pure compounds due to the simple, rapid, sensitive, and reproducible procedures involved⁹. The capacity of the leaf extracts to scavenge the stable 2,2'-diphenyl-2-picrylhydrazyl (DPPH) free radical was measured by extracting in 80% methanolic extract. To 0.1ml of freshly prepared cauliflower leaf extraction, 6ml of DPPH solution was added. The test tubes were kept in dark room at 35° for one hour. The optimal density of the sample and the blank was read spectrophotometrically at 517 nm. The readings were read in triplicates. Butylated hydroxytoluene was used as standard. The same procedure was followed in beetroot and radish leaves.

$$\text{The antioxidant capacity} = \frac{\text{Control} - \text{Test value}}{\text{Control}} \times 100$$

2. DETERMINATION OF TOTAL PHENOLS

Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry¹⁰. The Folin-Ciocalteu method is described in several pharmacopoeias¹¹. The leaf powders were treated with 80% ethanol for leaf extraction. To 0.5ml of freshly prepared samples each 8 ml of distilled water was added to all the test tubes. Later 0.5 ml of Follin's Ciocalteu Reagent added to all the tubes and kept under incubation at 40°C for 10 minutes. 1ml of Sodium Carbonate solution was added to all the test tubes. Then, the tubes were kept in the dark for incubation for one hour. The absorbance was read in triplicates using UV spectrophotometer at 660 nm using Gallic acid as standard. Readings were read in triplicates.

3. DETERMINATION OF TOTAL FLAVONOIDS

The spectrophotometric assay based on aluminium complex formation is one of the most commonly used procedure for the so-called total flavonoid determination, as the content of these compounds is considered as an important parameter for evaluating food or medicinal plant samples¹². One gram of each dried leaf sample was soaked in water over night. The contents were distilled in watt man filter paper. To 1.5mL of the leaf extract 3.5mL of distilled water and 0.3mL of 5% Sodium Nitrate was added to the tubes. After 5 minutes, 0.3mL of 10% Aluminum Chloride was added to all the tubes. At 6th minute, 2mL of 1M Sodium Hydroxide was added to mixture. Immediately, the contents of the reaction mixture were diluted with 2.4mL of distilled water and mixed thoroughly. Absorbance of the mixture was determined at 510nm versus a prepared blank immediately. Quercetine was used as the standard for quantification of total flavonoids. The readings were read in triplicates.

RESULTS

The role of free radicals in many ailments has been well established. Accumulation of free radicals may lead to various disease conditions. Much research confirms that foods or plants rich in antioxidants play an essential role in the prevention of free radical related diseases¹³.

Table I: Total Antioxidant Capacity

LEAF POWDERS	Total Antioxidants per 10mg
CAULIFLOWER	42.07 ± 0.95
RADISH	23.07 ± 0.31
BEETROOT	24.80 ± 0.72

Free radicals are inevitably produced in biological systems and also encountered exogenously, and are known to cause various degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing¹⁴. Antioxidants are the compounds, which combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process, viz., initiation, propagation and termination¹⁵. The results statistically reveal that the three leaves have the scavenging capacity to fight against the free radicals (Dpph) was observed among the three leaf powders. Cauliflower showed a good free radical scavenging capacity followed by beetroot and radish leaf powders.

Table II: Total Phenol

Leaf Powders	Total Phenols mg per 100 g
Cauliflower	349.50 ± 1.32
Radish	390.67 ± 12.86
Beetroot	118.83 ± 0.76

The significance of phenolic compounds has been analyzed mostly in plant kingdom among them *Brassica* species is well established. Statistical data reveal the presence of phenols in the leaf powders Phenols was seen higher in Radish and cauliflower leaf powders. Phenols in beetroot leaf powder were low comparing radish and cauliflower leaves.

Table III: Total Flavonoids

LEAF POWDERS	Total flavonoids g per 100 g
CAULIFLOWER	2.11 ± 0.06
RADISH	0.37 ± 0.02
BEETROOT	0.84 ± 0.07

Polyphenols (PPs) are a large and diverse class of compounds, many of which occur naturally in plants characterised by the presence of several phenolic groups. Naturally occurring polyphenols can be broadly divided into phenolic acids and flavonoids¹⁶. The presence of higher total flavonoid content in leaf suggests higher nutritional value of leaves, as flavonoids possess strong antioxidant activity and inhibit oxidative stress¹⁷. Supporting the above study total flavonoids was found to be higher in cauliflower leaf powder. Statistically contribution of flavonoids in cauliflower leaf powder was double compared to beet root and radish leaf powders. Thus the flavonoid content in these leaf powders

confirms the presence of polyphenols which is an essential bioactive compound for overall health.

DISCUSSION

The study reveals the significant presence of total antioxidants total phenols and total flavonoids in all the selected underutilized vegetable leaves. Statistically cauliflower leaves scored high in total antioxidant capacity comparing radish and beetroot leaves. Total phenols were marginally high in radish leaves comparing cauliflower leaves. Flavonoids were significantly high in cauliflower than radish and beetroot leaves. Hence the present study assures that these selected vegetable leaves that are easy to avail contain antioxidant activities.

CONCLUSION

The results of the study reveals the presence of bioactive compounds with reference to antioxidants, phenols and flavonoids which have positive effects on the health even though their contribution is in smaller amounts, it can play a very significant role in the health of humans. Cauliflower, radish and beetroot are less utilized leaves in our locality which are in need to be focused for human consumption. Due to lack of nutritional knowledge, negligence and its identity as animal fodder, these leaves are not included in our daily diet in spite of their easy availability and low economic cost. Hence its prime time to realize the benefits of these underutilized leaves which are packed with antioxidants that can prevent from all degenerative diseases and these antioxidant leaves can be treated as a natural preservative and in formulation of functional foods.

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