EVALUATION OF FLAVONOIDS IN SOME GREEN LEAFY VEGETABLES

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ABSTRACT:
Green leafy vegetables are a rich source of natural antioxidants. In the present paper a study of free radical scavenging activity was done in three plant species namely Ocimum Sanctum, Bacella alba var rubra and Murraya koenigii and the ability of the methanol extracts of these plants to scavenge the radicals generated by the in vitro systems and their ability to inhibit lipid peroxidation was studied by standard spectrophotometric methods and a positive correlation was established between some of these antioxidants and in vitro free radical scavenging activity of the plant extracts.

KEY WORDS: Green leafy vegetables, anti-oxidants, spectrophotometry.

1. INTRODUCTION:

Flavonoids are a large group of polyphenolic compounds with variable phenolic structures, possessing antioxidant properties both in vivo and in vitro systems (1, 2). Chemically flavonoids possess a fifteen carbon skeleton consisting of two benzene rings linked via a heterocyclic pyrene ring. They can be divided into a variety of classes such as flavones, flavonols and others. Flavonoids are phenolic substances isolated from vascular plants (3, 4). Flavonoids are the most abundant antioxidants in fruits and vegetables that reduce the incidence of chronic diseases, such as cardiovascular disease, diabetes, and cancer (5). These antioxidants possess radical scavenging and reducing properties and terminate the chain reactions caused by free radicals. Free radicals are generated by the body and are also present in the food we eat. Because of their high content of antioxidants, green leafy vegetables are one of the best cancer preventing foods (6).Oxidation occurs in the body which is accelerated by stress. Free radicals are formed, when certain molecules react with oxygen. These free radicals form chain of reactions which are damaging. Free radical damage to cells may initiate the early stages of cancer, damage to tissues and heart diseases (7). Antioxidants produced by the human body themselves get oxidised and combat these free radicals terminating the chain reactions thereby protecting the cells, thus waging war with the free radicals. . Antioxidants are reducing agents such as thiols, ascorbic acid and polyphenols, flavonoids .Flavonoids are phytonutrients which are powerful antioxidants found in almost all fruits and vegetables. Medicinal plants have therapeutic uses and the most important bioactive constituent of plants are antioxidants (8).

In the current trend the natural products rich in flavonoids or antioxidants are replaced by synthetic antioxidants to prevent the oxidative stress. Flavonoids have several health benefits and are associated with human dietary ingredients. The metabolism and biological activity of flavonoids depend on the total number of hydroxyl groups and functional groups about their nuclear structure. Some flavonoids possess antiviral activities (9).

2. METHODOLOGY

Flavonoids act in plants as antioxidants also exhibiting biological activities Flavonoids are good antioxidants reducing the free radical formation and scavenging the free radicals.

Preparation of vegetable samples:

The three green leafy vegetables shown in table-1 were studied, which were purchased from the local market of Jubilee hills, Hyderabad, India. The green vegetables were cleaned, washed, chopped into small pieces and air dried and ground to a fine powder.

Preparation of vegetable extracts:

Methanol extracts were prepared by soaking the plant materials in 1 lt. of methanol at room temperature. The extract was further filtered to remove any suspended impurities if present using whattman filter paper no: 42 .This pure extract was the stock solution from which several dilutions were made with distilled water to prepare the testing standards.

Determination of total flavonoid contents.

The total flavonoid content of the extracts was determined using spectro UV-Vis Dual Beam Model with 1 cm quartz cells. The extract was mixed with methanol, 20% $AlCl_3$ and potassium ethanoate and stirred evenly, then centrifuged at 3000rpm for 15-20 min. The absorbance was measured at 420nm using spectrophotometer and also the reagent blanks without the colour reagent.

3. RESULTS AND DISCUSSION:

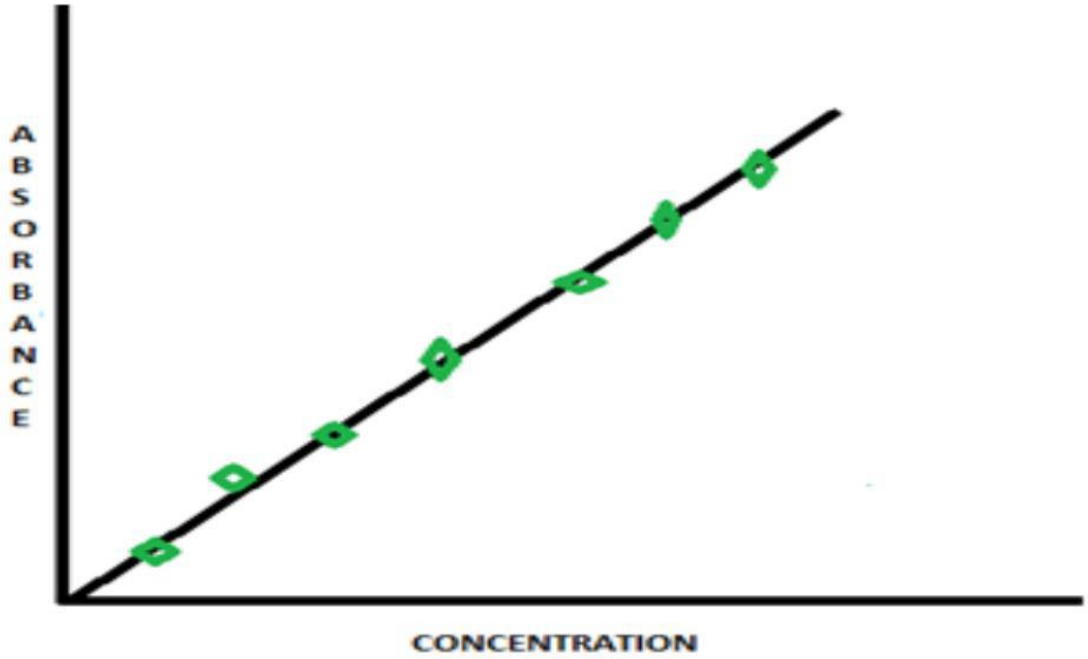
Spectrophotometric method was done based on flavonoid-aluminium chloride ($AlCl_3$) complexes.

Studies on flavonoids by spectroscopy revealed that most flavones and flavonols exhibit two major absorption bands at 320-385nm and at 250-285 nm corresponding to the two benzene rings. Functional groups attached to the flavonoid skeleton may cause a shift in the absorption.

The absorbance (ΔA) values of extraction were obtained by subtracting the absorbing values of blank from the absorbance (A) values of extraction and then the data of absorbance values were processed by the software .Calibration curves were constructed with 8 concentrations for all the above three mentioned plants (0.1,0.2,0.3,0.4, 0.5, 0.6, 0.7, 0.8 mg/mL). The calculation of linear regression was employed by the method of least squares. The accuracy was determined by the recovery test (10, 11).

TABLE-1

English Name	Botanical name	Family Name
Holy Basil	Ocimum Sanctum	Lamiacea
Malabar Spinach	Bacella alba var rubra	Basellaceae
Curry Leaves	Murraya koenigii	Rutaceae



Measurement of standard series- spectrophotometrically for calibrated plot

S.No.	Vol. of working standard (ml)	Concentration gm/ml	Double distilled water (ml)	Absorbance
1	1.0	0.001	22.0	0.324
2	2.0	0.002	24.0	0.456
3	3.0	0.003	25.5	0.567
4	4.0	0.004	26.7	0.674
5	5.0	0.005	28.2	0.774
6	6.0	0.006	29.2	0.821
7	7.0	0.007	29.8	0.945

4. CONCLUSION:

The green vegetables were analysed for the presence of flavonoids. The potential of green leafy vegetables as antioxidants for the presence of flavonoids was studied at multiple concentrations. The maximum scavenging activity and reducing power was exhibited by Murraya koenigii followed by Ocimum sanctum.

Murraya koenigii, Ocimum sanctum were found to have high antioxidant potency. The increased antioxidant potency of Murraya koenigii, Ocimum sanctum can be attributed to the presence of varying amounts of phytonutrients (12-14). The above analysis done spectrophotometrically was found to be much easier with reproducible results and less time consuming compared to other available procedures (15).

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