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EVALUATION OF UNDERUTILIZED VEGETABLE LEAVES AS A POTENT SOURCE OF DIETARY ANTIOXIDANT AND ANTIMICROBIAL AGENT

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ABSTRACT

The present study evaluated the antioxidant and antimicrobial potential of four under- utilized vegetable leaves namely carrot (*Daucus carota* (DC)), beet (*Beta vulgaris* (BV)), c*auliflower* (*Brassica oleracea* (BO)) and radish (*Raphanus sativus* (RS)). The total phenolic content (TPC), total flavonoid content (TFC) and total reducing power (TRP) were evaluated for the methanolic extract of leaves. DPPH radical scavenging activity, ABTS radical cation scavenging assay, hydroxyl radical (OH^-) scavenging activity, superoxide radical scavenging activity and antioxidant activity in linoleic acid emulsion system were carried out for the methanolic extracts of the samples to assess the *in vitro* antioxidant activities. The results showed that there exist a linear correlation between polyphenol content and antioxidant property. The methanolic extract of DC showed the highest phenolic content (34.2 mg GAE/g), DPPH radical scavenging activity (IC_{50} -32.5 µg/ml), ABTS radical scavenging activity (TEAC value – 46.47 µg/ml) and hydroxyl radical scavenging activity (IC_{50} -214.3 µg/ml). In addition to *in vitro* antioxidant activity, all the methanolic extract of these underutilized leaves possessed potent antimicrobial activity against many human pathogens.

Key words: Underutilized leaves, polyphenols, antioxidant, antimicrobial potential

INTRODUCTION

In all aerobic organisms, including human beings, there is a continuous production of reactive oxygen species (ROS) by normal metabolic processes or from exogenous factors and agents, which is balanced by antioxidant defence system. At physiological low levels, ROS function as "redox messengers" in intracellular signalling and regulation, whereas excess ROS induce oxidative modification of cellular macromolecules, inhibit protein function, and promote cell death (Circu et al., 2010). An imbalance between the production of ROS and the antioxidant defence system leads to oxidative stress. Oxidative damage accumulates during the life cycle and has been implicated in aging & age dependent diseases such as atherosclerosis, ischemic heart disease, cancer, diabetes, neurodegenerative diseases and other chronic conditions (Raghavendra et al., 2013). Antioxidant defence systems may prevent oxidative damages that occur due to ROS production by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Al-Azzawie &Mohamed, 2006) and therefore may either retard the progression of these diseases or may decrease the severity of the disease.

The synthetic antioxidants (butylated hydroxytoluene and butylated hydroxyanisole) which are commonly used in processed foods has been reported to contain certain side effects such as hepatotoxicity and carcinogenesis (Grice, 1986; Wichi, 1988). Therefore, potential sources of natural antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots (Ramarathnam et al., 1997). The ethnobotanical reports offer information on the medicinal properties of green leafy vegetables which include details on their antidiabetic (Kesari, Gupta, & Watal, 2005), anticarcinogenic (Khanna, Rizvi, & Chander, 2002), and antibacterial activities (Kubo, Fijita, Kubo, Nehei, & Gura, 2004). These beneficial effects of green leafy vegetables are attributed, at least in part to, antioxidants. Green leafy vegetables constitute a major part of any balanced diet and are good sources of minerals and vitamins (Raju et al., 2007). Leafy and green vegetables are most commonly consumed and are accessible throughout the year in India and other parts of the world.

The world is presently over-dependent on a few plant species. Diversification of production and consumption habits to include a broader range of plant species, in particular those currently identified as 'underutilized', can contribute significantly to improve health and nutrition, livelihoods, household food security and ecological sustainability (Oselebe et al., 2013). In particular, these plant species offer enormous potential for combating

against diseases. Hence, in the present study, attention has been devoted to the leaves of commonly available vegetables, which though underexploited in most cases, possess a tremendous potential to help people to overcome the deadly diseases of modern society. Among them, some of the underexploited leaves are carrot [*Daucus carota* (DC), beet (*Beta vulgaris* (BV), cauliflower (*Brassica oleracea* (BO) and radish (*Raphanus sativus* (RS)]. In addition, the plant extracts have been used to investigate the antimicrobial properties since they can be of greater significance in therapeutic treatments.

Therefore, the present study attempted to evaluate and compare the antioxidant and antimicrobial activity of the above mentioned leaf extracts.

MATERIALS AND METHODS

CHEMICALS

2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2, 2 Diphenyl-1-1-picrylhydrazyl (DPPH), quercetin, thiobarbituric acid (TBA), gallic acid, phenazine methosulfate (PMS) and catechin were purchased from Sigma–Aldrich (St. Louis MO, USA). Reduced Nicotinamide Adenine Dinucleotide (NADH), Folin-Ciocalteu reagent and sodium nitroprusside (SNP) are purchased from Sisco Research Laboratories Pvt Ltd (Mumbai, INDIA). All other chemicals and solvents used were of standard analytical grade.

PLANT MATERIAL

Selected plant material (*Beeta vulgaris, Brassia oleraceae, Rapanus sativus, Dacus carota-leaves*) were obtained from Central vegetable market, Trivandrum, Kerala, India. The leaves of all the plant materials were chopped, dried in oven at 60°C for 48 h and powdered (40-mesh). These powdered leaf samples (10 g) were extracted using soxhlet apparatus with methanol. The extraction process was repeated till the solvent became colourless. These solvent extracts were pooled, filtered through Whatman No. 1 filter paper and concentrated in vacuum at 40ºC using a Rotary evaporator (Heidolph LABORATA, Germany). Each extracts were made up to 100 ml using methanol and the residues obtained were stored at − 80ºC until further analysis.

DETERMINATION OF DRY WEIGHT OF THE EXTRACT

From methanol extract, 1 ml of sample was taken and placed into a previously weighed dried Petri plate. Sample was dried in oven at 120°C until a constant weight was obtained. The concentration in 1 ml of extract was calculated. Dry weight (µg) was calculated by the following formulae,

Dry weight (1 ml) =A-B

Where, A - weight of Petri plate with dry sample and B - weight of the Petri plate.

DETERMINATION OF ANTIOXIDANT ACTIVITY

The antioxidant capacity was assessed in terms of total phenolic content, total flavnoid content, total reducing power, DPPH radical scavenging activity, metal chelating activity, ABTS radical cation scavenging assay, hydroxyl radical scavenging activity and antioxidant activity in linoleic acid emulsion system.

TOTAL PHENOLIC CONTENT

Total phenolic contents were determined using Folin-ciocalteu reagent and expressed as gallic acid equivalents (GAE) (Singleton & Ross, 1965). The extracts were diluted with the same solvent used for extraction (methanol), to a suitable concentration for analysis and 0.5 ml of commercial Folin ciocalteu reagent was added. The contents were mixed well and kept for 5 min at room temperature followed by the addition of 1 ml of 20% aqueous sodium carbonate. After incubation at room temperature for one and half hour, the absorbance of the developed blue colour was read at 760 nm (Shimadzu UV-2450 Shimadzu corporation, Kyots, Japan) against reagent blank and the results were calculated as gallic acid equivalents (GAE) (mg/100 g) of sample, where gallic acid is used as the standard.

TOTAL FLAVONOID CONTENT

The total flavonoid content was determined by colorimetric method described by Joon *et al* (1999) with minor modification. Different concentrations of standard (quercetin) and extracts were prepared in methanol and 0.3 ml of 10% aluminium chloride was added to the mixture followed by 2 ml of 0.1N sodium hydroxide. The reaction mixtures were made up to 10 ml with distilled water. The mixtures were thoroughly vortexed and the absorbance was measured at 510 nm (Shimadzu UV- 2450 Shimadzu Corporation, Kyots, Japan). The concentrations of the samples were determined by plotting the values against the standard. The total flavonoid content of extracts was expressed as mg quercetin equivalents (QE) / g of extract.

TOTAL REDUCING POWER

Total reducing power (TRP) was estimated according to Oyaizu (1986). The principle lies on the reduction of potassium ferric cyanide to ferrous cyanide by antioxidants present in the sample. With the increase in antioxidants concentration, there is an increase in the reducing power, thus increasing the absorbance. Different concentration of the extracts (100-500 µg) and the standard, gallic acid, were mixed with 2.5 ml phosphate buffer (0.2 mM, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The reaction system was sealed and incubated at 50°C in a water bath for 30 min. Following incubation, 2.5 ml of 10% trichloroacetic acid was added to the assay system and the contents were mixed well. About, 2.5 ml sample was collected and mixed with 2.5 ml of distilled water and 0.5 ml of 0.5% ferric chloride. The color developed was read at 700 nm (Shimadzu UV- 2450 Shimadzu Corporation, Kyots, Japan) against reagent blank.

DPPH RADICAL SCAVENGING ACTIVITY

Free radical scavenging activity of the extracts were determined by using a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) following the method of Brand, Cuvelier & Berset (1995) and Abraham & Sindhu

(2006). DPPH is a free radical of violet colour. The change of colour from violet to yellow is proportional to the radical scavenging activity. The assay contained 1 ml of 0.05 mM DPPH in ethanol and various concentrations of extracts and standard (gallic acid). The contents were mixed well immediately and then incubated for 30 min at room temperature (24-30°C). The degree of reduction of absorbance was recorded in UV-Vis spectrophotometer at 517nm (Shimadzu UV- 2450 Shimadzu corporation, Kyots, Japan). The percentage of scavenging activity was calculated as:

$$\frac{(Ac - As)}{Ac} \times 100$$

Where, 'Ac' is the absorbance of control (without extract) and 'As' is the absorbance of sample.

Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain IC_{50} value. IC_{50} is defined as the amount of sample/standard required to scavenge 50% of free radical in the assay system. The IC_{50} values are inversely proportional to the antioxidant activity.

DETERMINATION OF ABTS RADICAL CATION SCAVENGING ASSAY

The experiment was carried out using an improved ABTS decolorisation assay (Abraham & Sindhu, 2006) which involves the generation of $ABTS^+$ chromophore by the oxidation of ABTS with potassium persulphate. The ABTS radical cations ($ABTS^{+}$.) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulphate (final concentration) and allowed to stand in the dark for at least 6 hours at room temperature before use. Absorbance at 734 nm was measured 7 min after the initial mixing of different concentration of the methanolic extracts (final concentration 10-40 µg/1.1 ml) with 1 ml of ABTS solution. The $ABTS^+$ scavenging capacity of the extract was compared with that of trolox which was used as a standard. A standard curve was prepared by measuring the reduction in absorbance of the $ABTS^+$ solution at different concentration of trolox and samples over a period of 7 min. The Trolox equivalent antioxidant capacity (TEAC) of an extract represents the concentration of trolox solution that has the same antioxidant capacity as the extract. The TEAC values were determined as follows:

ΔA trolox = (At = 0 min trolox – At = 7 min trolox) – ΔA solvent (0-6 min) (1)

ΔA trolox = *m* (Trolox), (2)

TEAC extract = (ΔAextract / *m*) .*d* (3)

where ΔA is the reduction of absorbance; A, the absorbance at a given time; *m*, slope of the standard curve; [Trolox], the concentration of trolox; *d*, the dilution factor.

HYDROXYL RADICAL (OH^-) SCAVENGING ACTIVITY

The sugar deoxyribose on exposure to hydroxyl radicals, generated by the Fenton reaction model system degrades in to fragments and generates a pink chromogen on heating with TBA at low pH (Halliwell, Guttridge & Aruoma, 1987). The reaction mixture, which contained various concentration of sample, deoxyribose (3.75 mM), H_2O_2 (1 mM), potassium phosphate buffer (20 mM, pH 7.4), $FeCl_3$ (0.1 mM), EDTA (0.1 mM) and ascorbic acid (0.1 mM), was incubated in a water bath at 37±0.5°C for 1 h. Further, 1 ml of TBA (1% w/v) and 1 ml of TCA (2.8% w/v) were added to the mixture and heated in a water bath at 100°C for 20 min. The absorbance of the resulting solution was measured at 532 nm (Shimadzu UV- 2450 Shimadzu Corporation, Kyots, Japan). Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain IC_{50} value.

SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Superoxide radical scavenging activity analysis was performed according to the method of (Parejo et al., 2002) using the xanthine-xanthine oxidase system. Xanthine is converted to uric acid by the enzyme xanthine oxidase with superoxide as a by product. This superoxide combines with nitro blue tetrazolium (NBT, 5 mg/ml buffer) and results in blue colour. If the tested sample contained antioxidant, it may scavenge the superoxide and thus the formation of blue colour is reduced. The reduction of colour is proportional to the antioxidant content in the sample and the blue colour developed was measured at 560 nm (Shimadzu UV- 2450 Shimadzu Corporation, Kyots, Japan). Briefly, 50µl xanthine and 20 µl of NBT were added to varying concentrations of extract and standard. Final volume was made up to 1 ml with phosphate buffer (50 mM, pH 7.5). Further, 50 µl of xanthine oxidase was added to system and mixed well to start the reaction and incubated at 37 °C for 30 min in water bath. The reaction was stopped after 30 min by adding 100 µl of 0.1N HCl. Absorbance of blank prepared without sample and standard was considered as 100% radical. Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain IC_{50} value.

ANTIOXIDANT ACTIVITY IN LINOLEIC ACID EMULSION SYSTEM

The antioxidant activity of the methanol extract was determined by the thiocyanate method (Duh et al., 1992). The samples in methanol at a concentration of 125 µg were mixed with linoleic acid emulsion in potassium phosphate buffer (0.02 M, pH 7.0). Linoleic acid emulsion (50 ml) was prepared by mixing and homogenizing 155 µl linoleic acid, 175 µl Tween 20 (as emulsifier) and 0.02 M phosphate buffer. The reaction mixture was incubated at 37°C. Aliquots of 0.1 ml were taken at various intervals during incubation. The degree of oxidation was measured by sequentially adding ethanol (5 ml, 75%), ammonium thiocyanate (0.1 ml, 30%) and ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl) to sample solution (0.1 ml) and the absorbance was read at 500 nm (Shimadzu UV- 2450 Shimadzu corporation, Kyots, Japan). Solutions without added extracts were used as blank samples. BHT is the positive control. The degree of oxidation was measured every 24 h and the data used are average of triplicate analyses. Percentage of inhibition of lipid peroxidation was calculated using the equation:

(Ac – As) x 100

Ac

DETERMINATION OF ANTIMICROBIAL ACTIVITY

Antibacterial activity was tested against two gram positive bacteria, *Micrococcus luteus* (MTCC 2470) and *Bacillus subtilis* (MTCC 2465) and one gram negative bacteria, *Pseudomonas aeruginosa* (MTCC 2453). Antifungal activity was tested against *Yarrowia lipolytica* (KACC 41237).

TESTING FOR ANTIBACTERIAL ACTIVITY

The well diffusion method as proposed by Pezer et al., (1990) was adapted for the present study with slight modification to assess the antibacterial activity of the extracts. Bacterial stock suspensions [(10^8-10^9) colony forming units (CFU) / ml] of 100 µl each was thoroughly mixed with sterile nutrient agar. Nutrient Agar inoculated with the test organisms were poured into sterile Petri plates. The agar was left to set in each of these plates for 1-2 h and wells of 10 mm in diameter were cut using a sterile cork borer. The total capacity of the well was nearly 0.5 ml and the extracts at a concentration of 300 µg were added to the wells. The plates were then incubated at 37°C in the upright position in room for 24 h. Control used was methanol. After incubation, the diameters of inhibition zones were measured. The activity of control was deducted from the samples and the results obtained were plotted.

TEST FOR ANTIFUNGAL ACTIVITY

The method similar to antibacterial activity was adapted for evaluating antifungal property of the extracts. Instead of nutrient agar, Potato Dextrose Agar was used. Antifungal activity was tested against *Yarrowia lioplytica*. 25 ml of suspension was poured in to the Petri dish. The inoculated medium was incubated at 25ºC in an incubator for two days and the zones formed were measured. The activity of reference was deducted from the test and the result obtained was plotted.

2.5. Statistical analysis

All the experiments were carried out in triplicates. The experimental results were expressed as mean ± standard deviation of triplicate measurements. Data were subjected to one-way ANOVA and the significance of differences between means was calculated by Duncan's multiple range test, using SPSS for Windows, standard version 16 (SPSS, Inc.), and significance was accepted at P ≤ 0.05.

RESULTS AND DISCUSSIONS

TOTAL PHENOLIC CONTENT

The total phenolic content of the extracts were determined by Folin-ciocalteau method. Table 1 shows the amount of phenolic content in four different extracts of vegetable leaves. The methanolic extract of DC had the highest phenolic content. The total phenolic content of DC, RS, BV and BO varied from 34.2 to 19.2mg GAE/g dry weight basis. The total phenolic content of extracts were in the order of DC> RS >BV >BO.

Studies show that when compared with carrot, carrot leaves shows higher phenolic content (Kaur et al, 2002). In plants, compounds or classes of phenolic compounds such as phenolic acids are responsible for antioxidant activity (Angelo & Jorge, 2007; Lako, 2007) and are present in vegetable leaves (Moon & Shibamoto, 2009; Fernandes et al., 2007).

TOTAL FLAVONOID CONTENT

Total flavonoid content in the leaves ranged from 28.1 to 51 mg QE /g of extract (Table 1). The flavonoid content was in the order BV> DC >RS >BO. Various studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables (Luo, Basile, & Kennelly, 2002).

Table 1: Determination of Total Flavonoid content and Total Phenolic content of the extracts

Samples	**Total phenolic content** (mg of Gallic acid equivalents / g)	**Total Flavonoid content** (mg of Quercetin equivalents / g)
DC	34.2 ± 0.9[a]	48.4 ± 0.7[b]
RS	31.8 ± 0.14[b]	35.6 ± 0.2[c]
BV	22.0 ± 1.8[c]	51.0 ± 0.2[a]
BO	19.2 ± 0.2[d]	28.1 ± 0.14[d]

Means in a row with the same letter(s) do not significantly differ at P<0.05 according to Duncans test.

TOTAL REDUCING POWER

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al, 1995). In this assay the yellow colour of the test solution changes to green depending on the reducing power of test specimen. The reducing power of the extracts and the reference compound, Gallic acid, increased steadily with their increasing concentration (Figure 1). The highest reducing power was exhibited by the methanolic extract of DC.

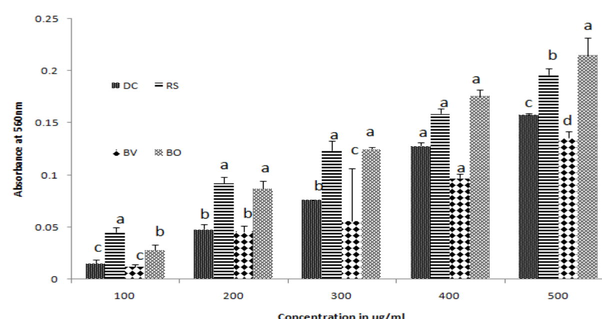

Fig. 1: Comparison of reducing power of the extracts with Gallic acid. Values followed by different letters were significantly different according to Duncan's multiple range test p < 0.05.

DPPH RADICAL SCAVENGING ACTIVITY

DPPH, a stable nitrogen centred free radical has been used to evaluate natural antioxidants for their radical quenching capacities in a relatively short time compared with other methods. The method is based on the reduction

of the absorbance of methanolic DPPH solution in the presence of a proton donating substance. The IC_{50} values of each extracts are represented in Table 2. Among the extracts, the DPPH radical scavenging capacity was highest for the methanolic extracts of DC and lowest for the methanolic extract of BO. The DPPH radical scavenging activity of these underutilized leaves was highly significant when compared with certain edible leafy vegetables reported by (Dasgupta et al, 2007). In the present study, the least DPPH radical scavenging activity among the underutilized leaves was demonstrated by BO extract (IC_{50} -128.9 µg/ml) which was significantly higher than the reported leafy vegetables, such as, *Bacopa monnieri* (IC_{50} -191 µg/ml), *Bauhinia racemosa* (IC_{50} -216 µg/ml), *Moringa oleifera* (IC_{50} -360 µg/ml), *Nyctanthes arbortristis* (IC_{50} -1946 µg/ml), *Trigonella foenum-graceum* (IC_{50} -847 µg/ml) (Dasgupta et al, 200 and Dharmalingam and Nazni, 2013) thus demonstrating the antioxidant potential of these underutilized vegetable leaves.

Table 2: IC_{50} values of DPPH, Superoxide, ABTS and Hydroxyl radical scavenging activities

	DPPH radical scavenging activity (IC_{50} in µg/ml)	**Superoxide radical scavenging activity** (IC_{50} in µg/ml)	**ABTS radical scavenging activity** (TEAC Value in µg/ml)	**Hydroxyl radical scavenging activity** (IC_{50} in µg/ml)
Standard (Gallic acid)	1.68	5.46	1	10.9
DC	32.5	29.20	46.47	214.3
RS	86.1	25.66	78.3	378
BV	39.7	41.63	118.2	649.6
BO	128.9	72.68	297	1168

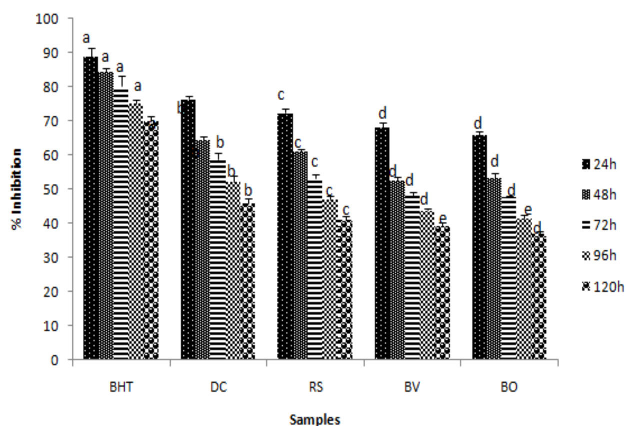

Fig. 2: Inhibition of Lipid peroxidation at a concentration of 125 µg. Values followed by different letters were significantly different according to Duncan's multiple range test $p < 0.05$.

ABTS SCAVENGING ACTIVITY

The experiments were carried out using an improved ABTS decolourization assay which involved the generation of ABTS chromophore by the oxidation of ABTS with potassium per sulphate. The relative antioxidant's ability to scavenge the radical $ABTS^+$ was compared to the standard, Trolox, which is an excellent tool for determining the antioxidants. Methanolic extracts of DC and RS possessed potent ABTS scavenging capacity among all the extracts (Table 2). Since the antioxidant activity is known to depend on the phenolic content, the methanolic leaf extracts of DC demonstrated highest ABTS radical scavenging capacity (IC_{50} value 46.47 µg/ml) which may be attributed to their higher phenolic and flavonoid contents.

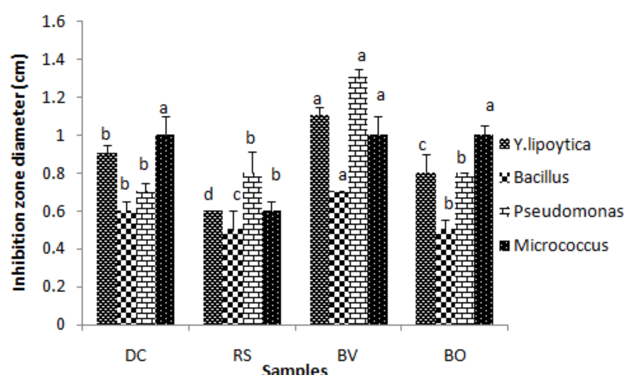

Fig. 1: Antimicrobial activity of extracts at 300 µg concentration. Values followed by different letters were significantly different according to Duncan's multiple range test $p < 0.05$.

HYDROXYL RADICAL SCAVENGING ACTIVITY

The antioxidant effect of several polyphenols that act as inhibitors of hydroxyl radical formation and lipid peroxidation has been correlated with ion chelating properties (Ohnishi et al; 1994). In the present study, the hydroxyl radical scavenging ability of the extracts when compared with the standard gallic acid was in the following order: DC> RS> BV>BO (Table 2). IC_{50} value was highest for the methanolic extract of DC (214.3 µg/ml). However, the underutilized BO leaf extract was having either comparable or significant higher hydroxyl radical scavenging activity than certain leafy vegetables reported by Dasgupta et al (2007) (*Bauhinia racemosa*, IC_{50}-1739 µg/ml; *Nyctanthes arbortristis*, IC_{50}-1782 µg/ml; IC_{50}-1269 µg/ml; *Trigonella foenum-graceum*, IC_{50}-1429 µg/ml).

SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Superoxide radical cannot directly initiate oxidative reaction but can accelerate the formation of hydroxyl radical which is the most reactive oxygen species. The methanolic extract of RS showed IC_{50} value of 24.66 µg/ml possessed significant superoxide radical scavenging activity compared to other extracts. The IC_{50} value for superoxide radical scavenging activity of four extracts compared with the standard gallic acid is shown in Table 2. All the underutilized leaves studied in the present

investigation demonstrated higher superoxide radical scavenging activity compared to the leafy vegetables reported by Das gupta et al (2007).

INHIBITION OF LIPID PEROXIDATION

Lipid peroxidation leads to rapid development of rancid and stale flavors and is considered as a primary mechanism of quality deterioration in lipid foods and oils (Guntensperger et al., 1998). The antioxidant effects of the extracts on the peroxidation of linoleic acid were investigated.

Methanolic extract of DC exhibited better activity among the four samples with 76.42 % inhibition of linoleic acid peroxidation at a concentration of 125µg and 24 hr time interval when compared with the positive control, BHT which shows 88.9% inhibition at similar concentration and time. The peroxidation inhibition of DC and BHT were found to be declined with time and reached 45.9% and 70.0 %, respectively, at 120 hrs. The lipid peroxidation inhibition activities of the samples are shown in the Fig. 2. The phenolic compound and other chemical components present in the extract may suppress lipid peroxidation through different chemical mechanisms including free radical quenching, electron transfer, radical addition and radical recombination.

ANTI MICROBIAL STUDIES

Antibiotic resistance is the ability of a microorganism to withstand the effects of antibiotics. Plant extracts and essential oils have been used as alternatives to antibiotic due to their antimicrobial activities and the favourable effect on the animal intestinal system (Al-Kassien, 2009). The Methanolic extracts of the leaves of DC, RS, BV, BO were subjected to preliminary screening for antimicrobial activity against three pathogenic bacteria *B. subtilis*, *M. luteus, P. aeruginosa* and one fungi *Y. lipolytica*.

A potent antimicrobial activity against the Gram-negative bacteria *P. aeruginosa* (13 mm) was observed in wells treated with BV extract. The activity shown by the methanolic extract of BV was comparable with the reported activity of leafy vegetables like *Mukia maderaspatana*, *Solanum trilobatum* (Sasipriya et al., 2012). The methanol extract of DC exhibited pronounced antimicrobial activity against *M. luteus* (10 mm) and the fungi *Y. lipolytica* (9 mm). The methanolic extract of RS also demonstrated antimicrobial activity against *P. aeruginosa* (8 mm) and *Y. lipolytica* (6 mm) which was significantly lower than the activity demonstrated by BV and RS extracts.

The highest antibacterial effect of methanol extract against these organisms may be due to the ability of methanol to extract some of the active properties in plants like flavonoids, phenolic compounds, saponins and other secondary metabolites which are reported for antibacterial activities (Cowman, 1999). Studies have also reported the presence of antibacterial substances such as sulphoraphene and raphanin in *Raphanus sativus* leaves (Preethi, & Jaspal, 2013).

It is worthwhile to note that the presence of moderate antioxidant activities in leaves (*Daucus carota*, *Beta vulgaris*, *Brassica oleracea* and *Raphanus sativus*), which are usually discarded as waste that can also be alternate sources of antioxidants. It is also quiet interesting to observe that the antioxidant activity of these underutilised vegetable leaves showed much better activity when compared with some leafy vegetables.

CONCLUSION

The present study investigated the antioxidant and antimicrobial potential of certain underutilized vegetable leaves using various *in vitro* studies. Methanolic extracts, rich in polyphenols and flavanoids showed potent antioxidant activity against various *in vitro* model systems. Our studies also proved direct correlation with antioxidant and phenolic/flavanoid content. In addition, all the methanolic extract of these underutilized leaves also possessed a remarkable antimicrobial activity against tested pathogens. Among all the organisms tested, the methanolic extract of *Beeta vulgaris* showed the highest antimicrobial potential.

Based on present study it is evident that underutilized leaves can be a potential source of natural antioxidant. The dietary intake of these leaves may help in defending against metabolic disorders arising directly or indirectly due to oxidative stress and also the infections caused by the tested pathogens.

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