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Antioxidant Activity in Methanolic Extract of Selected Vegetables

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ABSTRACT

Objective: Current research work focuses on antioxidant activity in selected domestic vegetables. In recent times, majority of the diseases and/or disorders are mainly associated to oxidative stress due to generation of free radicals. Phytochemicals are essential for the control and/or prevention of oxidation by antioxidants and leads to healthy life. Antioxidants are able to reduce the risk of degenerative diseases through quenching of free radicals. Recent research data showed that the quenching free radicals by solvent extract with different food sources. Therefore, the present research study focused on antioxidant activity in selected vegetables. **Materials:** Cashew, Gooseberry, Almond, Ginger, Mountain gooseberry, Carrot, Spinach, Coriander, Garlic were collected from the market of Tumakuru District of Karnataka State, India. **Methods:** Moisture and dry matter were estimated in vegetable by AOAC method. Antioxidants were extracted using methanol solvent through reflux and estimated by DPPH. **Results:** The present study results showed that Cashew (270µg), Gooseberry (133 µg), Almond (165µg), Ginger (5.6µg), Mountain gooseberry (152 µg), Carrot (219µg), Spinach (209µg), Coriander (155µg), Garlic (17µg) and Mint (5.7 µg) respectively. **Conclusion:** The present research investigation data showed that the marginal inhibition in Ginger, Garlic and Mint extracts. Antioxidant activity in selected vegetables was showed marginal inhibition in turn prevents or controls the degenerative diseases.

INTRODUCTION

The prevalence of degenerative diseases is showing high in both developed and underdeveloped countries due to today's lifestyle, food pattern, physical activity and stress. The free radicals and reactive oxygen species are generated from the oxidation of macromolecules such as proteins, lipids and nucleic acids leads to degenerative disease [1, 2]. The clinical research evidences showed that the consumption of fruits and vegetables can be controlled/prevent degenerative diseases due to free radical quenching capacity. Presently, 80 % of the world's population depends on medicinal plants to cure many diseases and 20% of medicinal plants have been submitted to pharmacological tests as therapeutic agents due to biological activities [5, 8]. The quenching of free radicals by the potential bioactive molecules present in the fruits and vegetables includes antioxidants, polyphenols, flavonoids, vitamins, minerals, amino acids and dietary fiber. Researchers have been showed that natural antioxidants may benefit to manage lifestyle diseases such as cancer, diabetes, coronary heart disease, cell damage and preservatives in food industry [1, 3, 4, 6, 7]. In addition, the research evidences showed that the bioavailability of bioactive molecules may control degenerative diseases, arthritis, inflammation, brain dysfunction and ageing process [2, 9-12]. Animal experiments were demonstrated by using nuts on the anticancer activity due to phytochemicals [8, 13]. The inhibition of lipoxygenase, prostaglandin synthase, cyclooxygenase was showed by flavonoids [14]. Currently, the bioactive molecules extraction is challenging in foods due to polar and non-polar with different solvents systems like ethanol, methanol, acetone, ethyl acetate [3]. Therefore, the present research paper focused on the antioxidant activity in selected commonly consumed vegetables from Tumakuru District of Karnataka state, India.

MATERIALS AND METHODS

Selected vegetables Cashew (*Anacardium occidentale*), Gooseberry (*Ribes uva-crispa*), Almond (*Prunus dulcis*), Ginger (*Zingiber officinale*), Mountain gooseberry (*Ribes montigenum*), Carrot (*Daucus carota subsp.sativus*), Spinach (*Spinacia oleracea*), Coriander (*Coriandrum sativum*) Garlic (*Allium sativum*) and Mint were collected from the local market of Tumakuru District of Karnataka State, India. The vegetable samples were brought to the laboratory. Cleaned, dried, powdered and stored for further analysis.

Chemicals

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) was procured from Sigma Aldrich. High purity chemicals were used in the present study unless otherwise specified. Prepared DPPH stock in methanol, the working concentration of DPPH in the assay was 0.14mM and stored in dark bottle at 4^{o}C. Ascorbic acid was used as reference standard for antioxidant activity.

Moisture content

The moisture content in vegetable samples determines the loss of water when heated in the hot air oven at specified temperature [15]. The water loss was measured before and after the drying process on percentage basis by thermogravimetric method as moisture content. Briefly, weigh dry clean, empty aluminum cup labeled appropriately (W). Weigh 5g of the sample in to the aluminum cup and note the exact total weight of cup with vegetable sample (W1). Keep it in the hot air oven at 100^{o}C for 2h. Cool it in desiccator and take the exact total weight of cup with dry sample. Repeat till difference of two successive weighing (W2).

Dry Matter (%) =W_2-W/ W_1-W X 100, Moisture (%) =100 - % Dry matter.

Fat extraction

The moisture free samples were used for the fat extraction by using petroleum ether (60-80^{o}C) in weighed dry round bottomed flask and weighed moisture free samples were in extraction thimble by using Soxhlet apparatus. The extraction fat was carried out at boiling temperature of the ether for 12h. The extract was concentrated through a complete removal of ether and the extract was stored in moisture free place.

Antioxidants extraction

According to Bushra Sultan [9] method Briefly, Fat free samples were used for the extraction of antioxidants with methanol solvent was carried out by using reflux condenser for 3 h at 60^{o}C. The extracts were concentrated after filter through Whatman filter paper and dry the extract then the sample was stored in dry dark place.

Antioxidants capacity

According to Brand-Williams [16] method briefly, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) oxidized form as stable radical with purple color; antioxidants can donate an electron to

DPPH present in the samples the purple color changes to colorless which was measured absorbance. Briefly, 860µl of 50% methanol with various concentration of vegetable extract, add 140µl of DPPH, mix and incubate at 37^{o}C for 30min. The absorbance was read at 517nm by spectrophotometer (Elico-SL159) against 50% methanol blank and control reaction was carried out by without test sample. IC_{50} values were calculated on the basis absorbance difference in the control and test sample. The antioxidant activity in the samples was compared with reference ascorbic acid standard.

Antioxidant inhibition = Absorbance of control - Absorbance of sample / Absorbance of sample *100

RESULTS AND DISCUSSION

Sample collection

The fresh selected domestic vegetables Cashew (*Anacardium occidentale*), Gooseberry (*Ribes uva-crispa*), Almond (*Prunus dulcis*), Ginger (*Zingiber officinale*), Mountain gooseberry (*Ribes montigenum*), Carrot (*Daucus carota subsp.sativus*), Spinach (*Spinacia oleracea*), Coriander (*Coriandrum sativum*), Garlic (*Allium sativum*) and Mint were collected from the market of Tumakuru district of Karnataka state, India.

Moisture and Dry matter content

The moisture content was very critical factor on the quality and shelf life of the foods in the food industries. The moisture content determine through the weight loss of substances mass when heated by the process of water vaporization. The sample substances mass is measured before and after the drying process for the moisture content determination on percentage basis by thermogravimetric method [17]. The present results showed moisture percent in Cashew, 3.12; Gooseberry, 14.4; Almond, 4.8; Ginger, 11.4; Mountain gooseberry,18.6; Carrot,10.4; Spinach, 8.5; Coriander,15.6; Garlic-39.5 and Mint,14.8 respectively (Table-1; Fig.1).The percent dry matter content was showed in Cashew,96.88; Gooseberry,85.6; Almond,95.2; Ginger,88.6; Mountain gooseberry,81;Carrot, 89.6; Spinach,91.5; Coriander,84.4; Garlic,60.5 and Mint,85.2 respectively (Table-1;Fig.1). The Garlic was showed high moisture and less dry matter content than Garlic, Spinach, Coriander, Almond, Carrot, Ginger, Mint, Gooseberry and Mountain gooseberry respectively. The Cashew was showed high dry matter and less moisture content compared to Spinach, Coriander, Almond,

Carrot, Ginger, Mint, Gooseberry, Mountain gooseberry and Garlic respectively. The percent moisture content of ginger was 11.4 compared to moisture content 10 [18]. The spinach leaves showed dry matter percent showed 91.5 compared to 93.76 [19]. The few earlier research values of moisture and dry matter were comparable.

Fat extraction

The moisture free samples were used for the fat extraction by using petroleum ether (60-80^{o}C) by using Soxhlet method. The extraction fat was carried out at boiling temperature of the petroleum ether for 12h. The concentrated extract was obtained after the removal of petroleum ether and extract used for the extraction of antioxidants. The research data showed that fat may interfere with the antioxidants activity. Therefore, fat extraction was performed in selected vegetables.

Antioxidants extraction

Fat free samples were used for the extraction of antioxidants with methanol solvent was carried out by using reflux condenser for 3h at 60^{o}C. The extracts were concentrated after filter through Whatman filter paper and dry the extract then sample was stored in dry dark place.

Antioxidant activity

Many researchers have been showed natural antioxidants are known to prevent many diseases. Oxidative stress appears to be an important element to many human diseases. The oxidation of biomolecules was essential to sustain life and may damage cells. Hence, antioxidants may require for maintaining complex systems such as ascorbic acid, glutathione, vitamin-E and enzymes catalase, superoxide dismutase and peroxidases [9]. Reactive oxygen species are produced during metabolism leads to many disorders and diseases. The endogenous antioxidant acts as defense mechanism during normal cell respiration against reactive oxygen species and also natural antioxidants supplement through the diet as plant sources [9]. Antioxidant activity and inhibition was estimated by DPPH as stable radical with purple color changes to colour less after electrons received by the methanol extract can be measured absorbance at 517nm by using spectrophotometer and compared with ascorbic acid reference standard (Fig.2). The purple color represents the DPPH and less color represents the presence of antioxidants in the vegetables which donates electrons. The inhibition

concentration was calculated on the basis of absorbance difference in the control and test sample. The present research investigation results showed IC_{50} value of Cashew, 270 µg; Spinach, 209 µg; Coriander, 155 µg; Almond, 165 µg; Carrot, 219 µg; Ginger, 5.6 µg; Mint, 5.7 µg; Gooseberry, 133 µg; Mountain gooseberry, 152 µg and Garlic, 17 µg respectively (Table-1; Fig.3). The present research investigation results also showed the percent inhibition of Cashew, 80; Spinach, 83; Coriander, 90; Almond, 80; Carrot, 70; Ginger, 75; Mint, 80; Gooseberry, 93; Mountain gooseberry, 75 and Garlic, 60 respectively (Table-1, Fig.4). Highest antioxidant activity was showed in mint, ginger and garlic.

Table No. 1: Moisture, Dry matter, IC_{50} value and inhibition in vegetables

Sample	Moisture (%)	Dry matter (%)	IC_{50} (µg/ml)	Inhibition (%)
Cashew	3.12	96.9	270	80
Spinach	8.50	91.5	209	83
Coriander	15.6	84.4	155	90
Almond	4.80	95.2	165	80
Carrot	10.4	89.6	219	70
Ginger	11.4	88.6	5.6	75
Mint	14.8	85.2	5.7	80
Goose berry	14.4	85.6	133	93
Mountain goose berry	18.6	81.4	152	75
Garlic	39.5	60.5	17	60

Moisture: t-value 4.396, $p < 0.005$, Antioxidant capacity: t- 3.923 $p < 0.005$, Percent inhibition : t- 26.226, $p < 0.005$

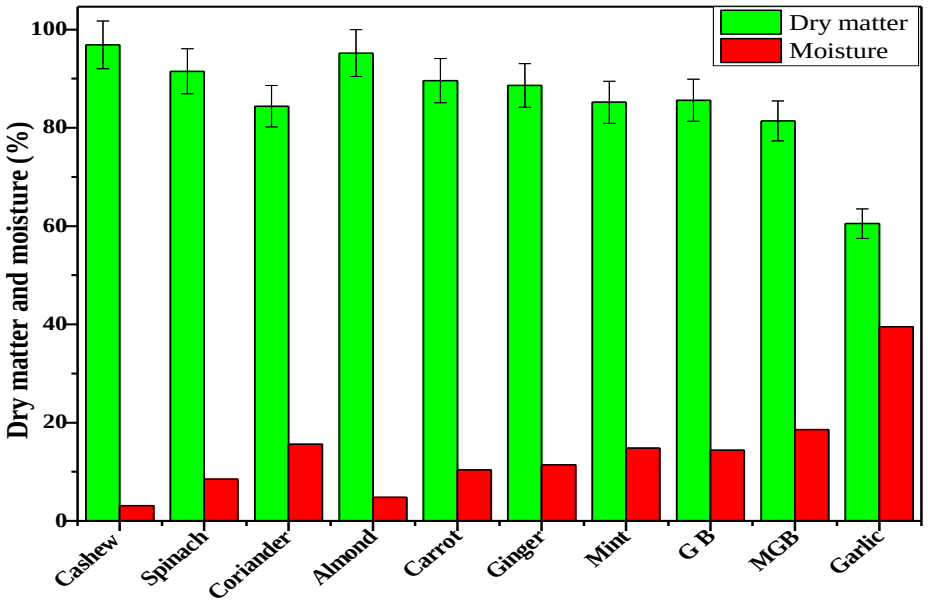

Figure No. 1: Bar diagram showing moisture and dry matter content in selected vegetables.

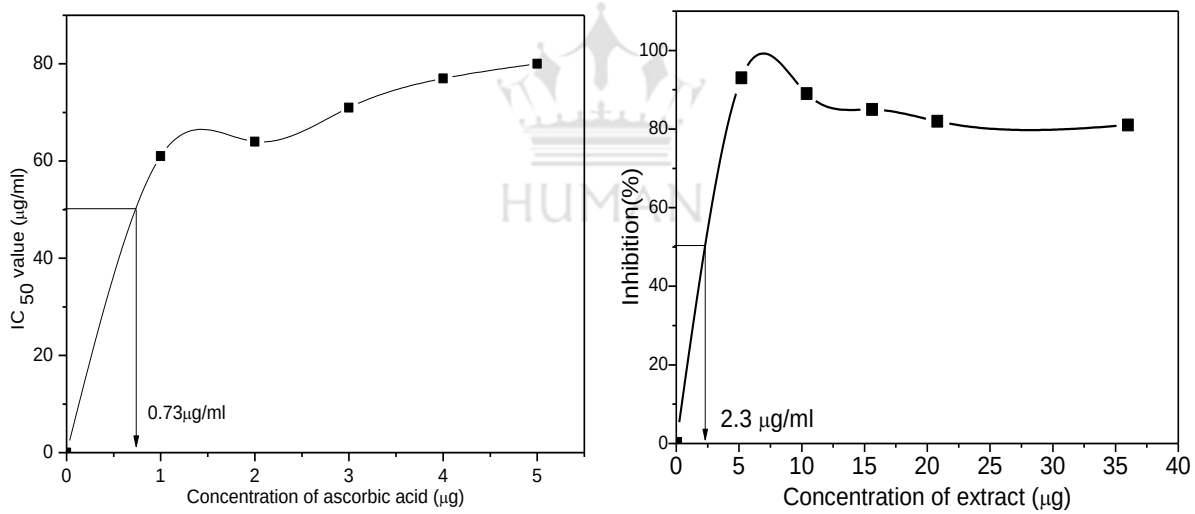

Figure No. 2: Graphical representation of IC_{50} value of Ascorbic acid and Gooseberry

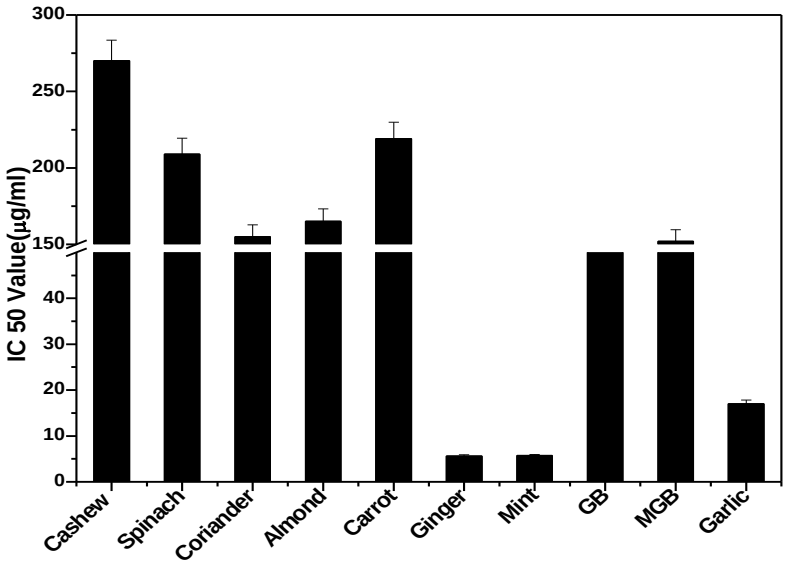

Figure No. 3: Antioxidant capacity as IC_{50} (µg/ml) in selected vegetables

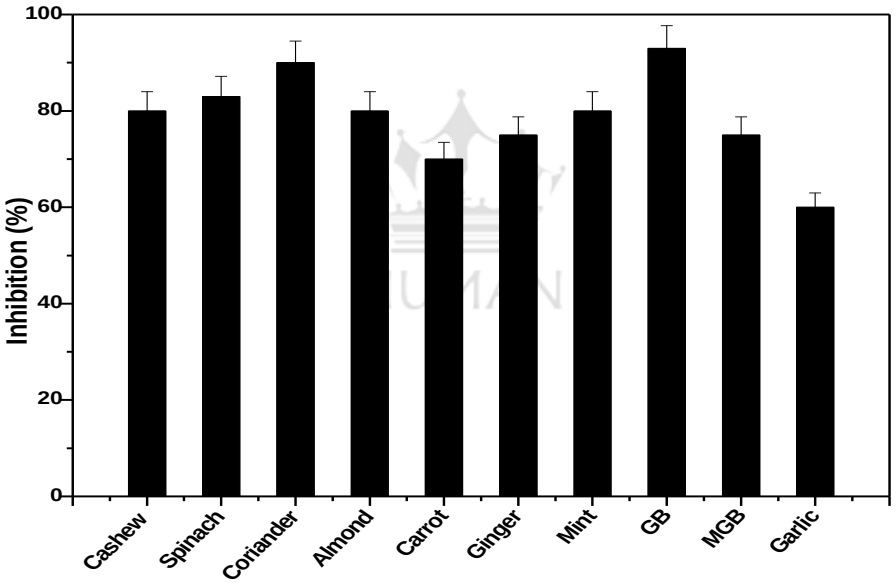

Figure No. 4: Percent inhibition in selected vegetables

Present study indicated that the antioxidant activity in ginger extract was 5.6 µg/ ml compared to 4.35 mg/ml [20]. The antioxidant activity of gooseberry was 133µg/ml but recent data exhibited that the gooseberry pulp and seed was 5.96µg/ml and gooseberry 6.79µg/ml respectively [8]. Earlier report showed that higher antioxidant activity of methanol extract in carrot peel [21]. Further, recent data showed potent scavenger activity in almond hull and shell and from different varieties of almond showed 12.15 to 18.22 % in 80 % methanol extract [7, 13]. In the present study inhibition of almond methanol extract was

showed 80 % inhibition compared to 47. 69 % and 91.3% [3, 22] and 34.91 % in methanol extract of almond peel [21]. Whole carrot methanol extract was showed 70 % compared to the results showed that the scavenging activity was 96.95 % in acetone [21]. In mountain, gooseberry showed 75% of scavenging activity compared to 72 % [18]. Gooseberry inhibition was 93% compared to 87 % and gooseberry seed extract was 90 % [8]. Coriander leaves showed 90 % inhibition compared to 62.2 % [10]. Spinach leaves showed inhibition 83% compared to 96.1 [19]. The present research investigation of antioxidant activity in selected vegetables Gooseberry, Mountain gooseberry, Spinach, Coriander, Mint, Almond, Cashew, Carrot, Ginger and Garlic extracts. The results showed that the ginger, mint, garlic are showing maximum antioxidant capacity among selected vegetables. The present investigation revealed that solvent methanol extracts of selected foods prepared by reflux extraction method exhibited good antioxidant activities. The free radical scavenging activity of gooseberry and spinach extract found to be marginal quantity in methanol extract compared to other vegetable extracts. The present data would certainly help to establish the potency of the selected vegetables as potential sources of natural antioxidants to be used for neutraceutical and functional food applications. The create awareness about the natural ingredients in the plant sources for safe consumption vegetables to improve and/or prevent many diseases.

CONFLICTS OF INTEREST

The data and research results are honest and the author reports no conflicts of interest in this work.

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