

Evaluation of phytochemical and *in vitro* antioxidant activity of water and acetone extracts of selected vegetables

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Abstract: The aim of this study was to determine antioxidant activity of *Brassica oleracea* (Cabbage), *Brassica rapa* (Turnip) and *Raphanus sativus* (Radish). All of the three selected vegetables were ultrasonically extracted in water and acetone. The antioxidant activity was determined by DPPH and H₂O₂. Highest phenolic, flavonoid and flavonol contents were found among water extract of *Raphanus sativus*, *Brassica oleracea* and *Brassica rapa* respectively. Highest %age of DPPH inhibition was exhibited by water extract of *Brassica rapa* and highest OH[•] radical scavenging activity was shown by water extract of *Raphanus sativus*.

Keywords: *Raphanus sativus*, *Brassica oleracea*, *Brassica rapa*, acetone, antioxidant activity.

INTRODUCTION

Naturally, vegetables possess significant amounts of bioactive compounds. Many evidences recommend that for human health, consumption of a diet rich in vegetables and fruits has positive implications. In the last few years, special attention has been diverted to those plants which are considered rich in phytochemicals. Now-a-days, it is assumed that due to the high levels of carotenoids, tocopherols and ascorbic acid in cruciferous vegetables, these act as a good source of natural antioxidants. Because of the nutritional value of *Brassica* species, the popularity and consumption of vegetables is increasing. *Brassica* foods are rich in nutrition, thus provide nutrients and health-promoting phytochemicals such as carotenoids, vitamins, minerals, fibre, soluble sugars, phenolic compounds and glucosinolates (Podsedeck 2007; Jahangir *et al.*, 2009)

One of the major agricultural products which contain high level of phytochemicals including minerals, flavonoids, glucosinolates and vitamins is *Brassica oleracea* (Cao *et al.*, 1996). Vitamins and flavonoids are the main antioxidative constituents present in *Brassica oleracea*. It helps in fight against cancer and reduced cardiovascular diseases. All over the world, one of the oldest cultivated vegetables is *Brassica rapa* and is being used for human consumption (Liang *et al.*, 2006). In *Brassica rapa* different components such as phenolic compounds, flavonoids and several phenolic acids were determined by high performance liquid chromatography-diode-array detector (HPLC-DAD). To cure human diseases including poor digestion and liver dysfunction, *Raphanus sativus* have been used as medicinal food (Gutierrez and Perez 2004). Recent studies show that extracts of *Raphanus*

sativus have different biological activities such as antiproliferative effects (Beevi *et al.*, 2010) antioxidant (Wang *et al.*, 2010), induction of detoxification enzymes and antimutagenic. *Raphanus sativus* contains many phytochemicals including phenols and anthocyanins.

Flavonoids are the fraction of naturally occurring of about 4000 polyphenolic compounds with C₆-C₃-C₆ backbone, which are found universally in foods of plant origin (Harborne 1986). These are familiar as pigments which are liable of colors of leaves. Flavonoids are extensively strewn in fruits, vegetables, seeds, herbs, nuts, stems, flowers, red wine and tea. Polyphenols represent the main bioactive phytochemicals that have been implicated in anticipation of many diseases like cancer and diabetes (Asami *et al.*, 2003). Flavonoids are structurally categorized into five types; flavones, flavanones, flavonols, flavan-3-ols and anthocyanins (Peterson and Dwyer 1998).

Plant phenolics are secondary metabolites with broadly spread chemical nature and potential including flavonoids, tannins, coumarins, lignans, xanthones and stilbenes (Liu and R.H., 2004). In addition to their biological, nutraceutical and clinical effects (Maimoona *et al.*, 2011), flavonoids are drawn in many resistance mechanisms. These bioactive security compounds are also responsible for plant responses to environmental hazards, such as temperature variations, air pollution and UV radiations. Phenolic compounds are habitually fabricated by commencement of metabolism caused by accidental fire or prescribed burning (Alonso *et al.*, 2002, Cannac *et al.*, 2007).

Flavonoids are present in many plants and have spacious actions. Due to diverse properties of flavonoids like

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antioxidant (Cazarolli *et al.*, 2008), anti-inflammatory, anti-allergic, antimutagenic, antiviral (Friedman 2007), antibacterial (Cushnie and Lamb 2011), antineoplastic and antithrombotic, they have many health reimbursement and pharmacological effects (Middleton *et al.*, 2000). Flavonoids help in UV filtration, nitrogen fixation and floral pigmentation in higher plants. They are also drawn in many functions and also operate as chemical messengers, physiological regulators and cell cycle inhibitors. Flavonoids are present in parsley, citrus fruits, leafy vegetables, cabbage, tomatoes, onions (Spencer and Jeremy 2008), blueberries (Radojevic and Bashkin 1999), blackberries, black tea, green tea and bananas. Foods prosperous in flavonoids alleviate many diseases like shield of blood vessels from leakage, increase the power of vitamin C, defend cell from oxygen damage and avoidance of inflammation of body. Flavonoids help to put off following diseases; diabetes, allergy, asthma, gout, cataracts, Candida infection, stomach ulcer and hemorrhoids.

Deficiency symptoms of flavonoids are nose bleeds, inflammation after injury, hemorrhoids and feeble resistant function. Even high prescribed amount of flavonoids has no unsafe effect. There are many factors which have an effect on the flavonoids. Heating, level of acidity and extent of processing greatly affect the contents of flavonoids. Heating removes flavonoid content, for example, boiling of fresh spinach exclude 50% of total flavonoid content and boiling of onion exclude 30% of total flavonoid content. Overcooking of vegetables also removes lot of nutrient contents.

MATERIALS AND METHODS

Collection of vegetables

Three samples of vegetables (name of samples) were collected from Bhimber City, Azad Jammu and Kashmir. These vegetables were washed with tap water and then rinsed with distilled water. The collected samples were cut into small pieces, dried at room temperature and grinded into powdered form.

Extraction

One g of powdered sample of vegetables was taken in a centrifuged tube and added 10mL of water. Centrifuged the mixture at 6000rpm for 15 minutes. This process was repeated thrice. Supernants were collected with the help of pipette. Same process was repeated for acetone extracts.

Phytochemical screening

Phytochemical study was carried out in order to determine flavonoids, flavonols and phenolic contents.

Flavonoids

Flavonoids were analysed by colorimetric method (Lin and Tang 2007). Five mL of water and acetone extracts were taken in test tubes, and each was added 0.3mL of 5%

sodium nitrite solution. After five minutes, added 0.3mL of 10% aluminium chloride solution in the given mixture. Reaction was stopped after 6 minutes by adding 2mL of 1M sodium hydroxide solution. The given mixture was diluted upto 10mL with distilled water. The absorbance was immediately measured at 510nm. Rutin was used as standard in order to determine flavonoid contents.

Flavonols

Flavonols were determined by method described by (Kumaran and Karunakaran2006). Two mL each of water and acetone extracts were taken in separate test tubes and added 2mL of (50g/L) sodium acetate and 2mL of 2% aluminium chloride in the extracts. The mixtures were allowed to stand at room temperature for 2.5h. Absorbance was measured at 440nm after 2.5h. Flavonol contents were expressed as rutin equivalents.

Phenolic contents

The analysis of phenolic contents was done with the help of method described by Lin (Lin and Tang 2007). One mL each of water and acetone extracts was taken in separate test tubes and added 5mL of 10 times diluted Folin ciocalteu and 4mL of 7.5% sodium carbonate solution in each of the extracts. Before measuring the absorbance the mixtures were allowed to stand at room temperature for 90 minutes. After 90 minutes, the absorbance was recorded at 760nm. Gallic acid was used as standard for the determination of phenolic contents.

Antioxidant activity

Antioxidant activity was carried out in order to determine DPPH and OH radical scavenging activity.

DPPH scavenging activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity was detected by the method described by Yu and Aoshima (Yu *et al.*, 2002, Aoshima *et al.*, 2004) with a little changing. About 1.0mL of the sample extract was taken in the test tube and added 2.5mL of (0.1mM in methanol) DPPH into the extract and incubated the mixture at room temperature in dark for 30 minutes. At 517nm, disappearance of color of DPPH was measured against blank. Results were calculated as Gallic acid equivalents and % inhibition was measured by following relationship:

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of extracts of vegetables in water and acetone has been described by Yu *et al.*, (2004). About 3mL of water or acetone extract was taken in a test tube. Added 1mL of 1, 10-phenanthroline (0.04 M), 2.0mL of 0.2 M phosphate buffer, and 0.04mL of ferrous sulphate (0.02M) into the extract. The reaction was started by the addition of 0.1mL of 7mM H₂O₂ in the given mixture. The mixture was incubated at room temperature for 5 minutes and then measured the

absorbance at 560nm. The hydroxyl radical scavenging activity was expressed as:

RESULTS

Total phenolic and flavonoid contents

Flavonoid contents in selected vegetables are shown in fig. 1, which depicted the highest concentration (770 mg/g) of flavonoids in water extract of *Brassica oleracea* and in acetone extract of *Raphanus sativus* (349.58mg/g). Flavonoid contents were found comparable in water extract of *Brassica rapa* (434.8mg/g) and *Raphanus sativus* (458mg/g) (table 1).

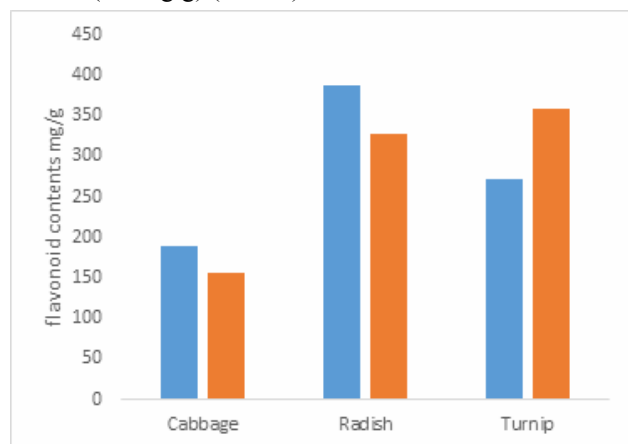


Fig. 1: Comparison of total flavonoid contents.

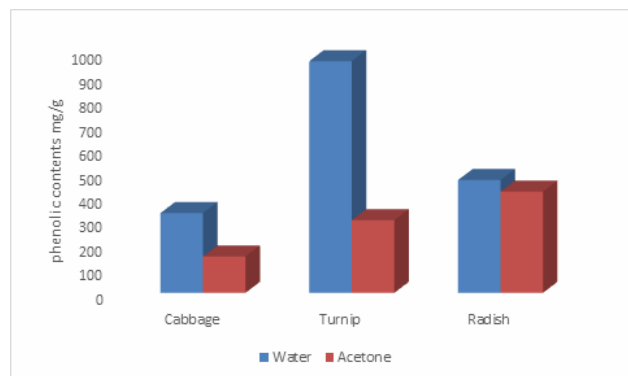


Fig. 2: Comparison of total phenolic contents.

In given vegetables, highest total phenolic contents (fig. 2) were recorded in water extract of *Raphanus sativus* (386.5mg/g) and acetone extract of *Brassica rapa* (358.5 mg/g). Lowest values were shown by water extract of *Brassica oleracea* (188.5mg/g) and acetone extract of *Brassica oleracea* (155.5mg/g). Phenolic contents of *Raphanus sativus* in water extract were recorded as 368.5 mg/g and 328.16mg/g in acetone extract.

Total flavonol contents

Fig. 3 shows the flavonol determination in vegetables and reveals highest flavonol contents in *Brassica rapa*, both in water (493.16mg/g) and acetone extracts (853.5mg/g) followed by the *Raphanus sativus* in water extract (488.16

mg/g) and *Brassica oleracea* in acetone extract (599 mg/g). Least concentration of flavonols is shown by water extract of *Raphanus sativus* (368.12mg/g) (table 1).

DPPH scavenging activity

Highest DPPH scavenging activity was shown by *Brassica rapa* (90.1%) in water extract, followed by *Raphanus sativus* (86%) (fig.4, table 2). Lowest DPPH scavenging activity was shown by water and acetone extracts of *Brassica oleracea* (85.1% each). In acetone extracts of waste water vegetables, highest DPPH activity was shown by *Raphanus sativus* (92.5%), followed by *Brassica rapa* (85.84%).

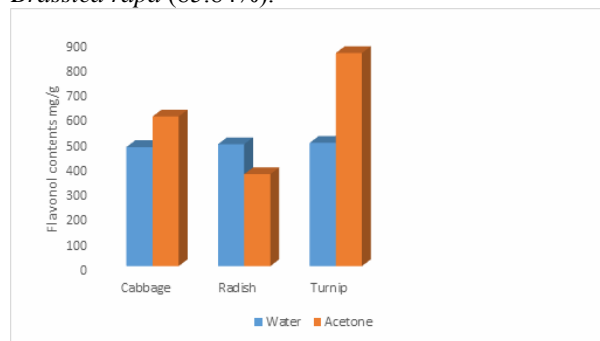


Fig. 3: Comparison of total flavonol contents.

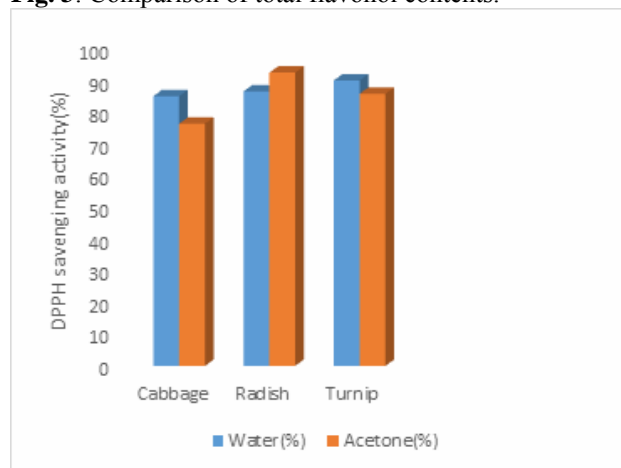


Fig. 4: Comparison of DPPH scavenging activity.

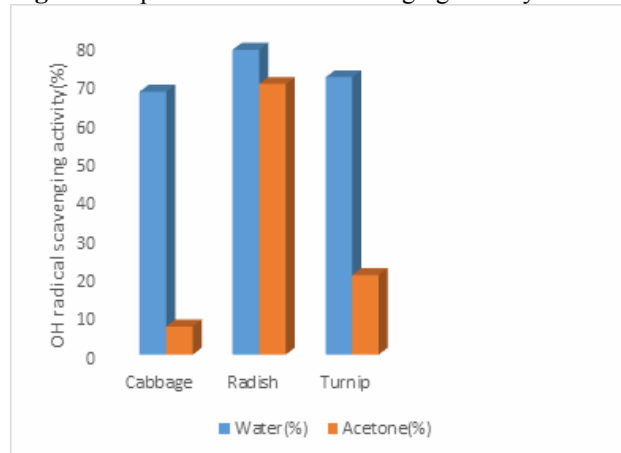


Fig. 5: Comparison of OH radical scavenging activity.

Table 1: Phytochemical constituents of vegetables

| Vegetable | Total phenolic contents (mg/g) | | Flavonoid contents(mg/g) | | Flavonol contents(mg/g) | |
|--------------------------|--------------------------------|-----------------|--------------------------|-----------------|-------------------------|-----------------|
| | Water Extract | Acetone Extract | Water Extract | Acetone Extract | Water Extract | Acetone Extract |
| <i>Brassica oleracea</i> | 188.51 | 155.51 | 770.00 | 199.80 | 476.50 | 599.00 |
| <i>Raphanus sativus</i> | 386.5 | 328.16 | 458.00 | 349.80 | 488.16 | 368.12 |
| <i>Brassica rapa</i> | 271.5 | 358.50 | 434.80 | 151.50 | 493.16 | 853.50 |

Table 2: Antioxidant activities of vegetables

| Vegetable | DPPH scavenging activity (%) | | OH radical scavenging activity (%) | |
|--------------------------|------------------------------|-----------------|------------------------------------|-----------------|
| | Water Extract | Acetone Extract | Water Extract | Acetone Extract |
| <i>Brassica oleracea</i> | 85.09 | 76.4 | 67.97 | 7.28 |
| <i>Raphanus sativus</i> | 86.60 | 92.5 | 78.81 | 69.94 |
| <i>Brassica rapa</i> | 90.06 | 85.84 | 71.67 | 20.57 |

Hydroxyl (OH) radical scavenging activity

OH radical scavenging activity of selected vegetables is shown in fig. 5. In acetone extracts, all vegetables analysed were found good OH radical scavengers with inhibition above 50%. Highest scavenging activity was recorded for water extract of *Raphanus sativus*, while the lowest scavenging activity was observed in *Brassica oleracea* and *Brassica rapa*. In case of acetone extract, all vegetables analysed were found weak OH radical scavengers with lower than 50% inhibition except *Raphanus sativus* (69.94%). The highest scavenging activity was recorded for acetone extract of *Raphanus sativus* (69.94%) and lowest scavenging activity for acetone extract of *Brassica oleracea* (7.28%) (table 2).

DISCUSSION

Phytochemicals are the compounds which occur in plants naturally and have good antioxidant potential. These compounds work with fibers and nutrients to protect humans against different diseases. They are secondary metabolites produced by the plants and are used extensively in food additives, drugs, flavours, fragrances and pesticides. At present, these compounds have found frequent application in clinical usage. Owing to the significance of the phytochemicals, the scientists are working continuously to augment their fabrication inside the plants by diverse techniques. Vegetables and plants are considered affluent source of free radical scavengers. They restrain antioxidants, which can retard or terminate the rate of oxidation reactions and are associated with agents of more than hundred diseases and ageing as well. Phenolics are the major group of phytochemicals which are accountable for antioxidant activity of plants. Oxidative stress is reduced by flavonoids which scavenge peroxy radicals efficiently due to their good reduction potentials relative to alkylperoxy radicals. Hence, flavonoids are thought to be effective reducers of lipid peroxidation. Hydrogen donating ability of flavonoids is due to presence of B-ring catechol group which enhance scavenging of reactive radical species.

Anthropological population is increasing at the rate of 10 billion per year that could touch ten billion by the year 2050. In order to provide adequate nutrition to each individual, different plans are required to be developed. The staple problem in the production of crops is the ailment of crops caused by weeds and pests. Numerous phytochemicals are produced by the medicinal plants which insure their survival by protecting them against infectious diseases (Ciccio G *et al.*, 2000). The results obtained in our study are in good agreement with former studies on plants regarding their phytotoxicity (Nisar M *et al.*, 2009).

CONCLUSION

It is concluded from the present study that water extracts of all vegetables have high flavonoid, flavonol and phenolics contents except *Brassica rapa* which shows high phenolic and flavonoid contents in acetone extract. In addition water extracts of vegetables show greater DPPH and OH radical scavenging activity than acetone extracts of vegetables. Present study reveals that extracts are effective in treating various diseases which are caused by free radicals. Furthermore, the frequent use of *Brassica oleracea*, *Brassica rapa* and *Raphanus sativus* is suggested to minimize the toxic effects of free radicals produced as a result of disorders in human metabolism.

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