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Comparative Study of Antioxidant in Radish (*Raphanus Sativus L.*) At Different Areas of District Khairpur Mir's

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ABSTRACT

The sample of three varieties of Radish (TakeeJapane, Neutech, F-1 Hybrid) was collected during the winter season from various parts of the district of Khairpur Mir's. The physical properties of each sample, like moisture content and PH etc., were determined immediately after collection within 24 hours. Extraction was done using three types of solvents i-e methanol, water, and methanol: water (1:1) separately. Using a double beam spectrophotometer, each Variety's free radical scavenging efficacy was evaluated in 2, 2-diphenyl-1-picrylhydrazyl (DPPH). After that, half-maximal inhibitory concentration IC50 of each property was calculated. The IC50 value of each Variety was found very close to (58.92) µg/ml standard ascorbic acid indicating that Radish is a good antioxidant. The observed IC50 values of the three varieties of the Radish was found in the range of 59.688 to 66.088 µg/ml (TakeeJapane), 44.229 to 68.347 µg/ml (Neutech) and 31.644 to 57.65 µg/ml (F-1 hybrid). The data indicate that IC50 values F-1 hybrid Variety were lowest than other two varieties indicating better antioxidant.
Keywords: Free radical, Radish (*Raphanus Sativus L.*), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), IC50, Scavenging.

Introduction

Vegetables are considered a prerequisite in phytochemicals and dietary fibre a nutrient. According to research, vegetables are multivitamin carriers that contain vitamins A, C, E, K, and other phenolic resin-based substances. Due to the inverse relationship between vegetable consumption and the presence of various heart diseases, gut diseases and coronary heart disease (CHD) must rise to the surface, according to contradictory data and correlations. (Ashrafe *et al.*, 2016). Consumption of vegetables and fruits as a source of minerals and vitamins helps to ensure a balanced and nutritious diet (Sarker, U. and Oba, S, 2020). Phytochemicals found in fruits and vegetables work as favourable factors for the human body through various processes (Biondi, F.et al., 2021). Comprehensive epidemiological studies have revealed highly inverse results for deporting concerning the utilization of various vegetables and fruits; additionally, they have been implicated as anti-blocking agents in the development of several disorders, including coronary artery disease, cancer, oedema, and ageing, because they contain therapeutic agents, the use of plant vegetables is beneficial for the treatment of various diseases all over the world. (Kala *et al.*,2006). They contain most of the nutrients that humans regularly require for illness prevention. (Holman & Meyers, 2005). These nutrients are typically found in plants and vegetables (Greathead et al., 2003). Vegetables are also high in organic chemicals, which are engaged in cell strengthening and may be a factor in the decrease of cancerous growth and cardiovascular disease. (Yahia *et al.*,2009). According to medical research, vegetables include a variety of vital components that play an essential role in cancer prevention. (Halliwell *et al.*,1997; Wiseman and Halliwell, 1996). Modern research specifies that Mammalian cells and animals are made up of many different types of essential polyphenol mixture that comes from ground vegetables (W.H.O.2011). Polyphenols are the most common antioxidants found in plants, made up of substructures. Butylated hydroxyanisole, propyl gallate, and butylated hydroxyl toluene are cancer-fighting antioxidants present in fruits, vegetables, and other plants. (House, Robbins and Metzner, 1982). Several studies have suggested that the number of phytonutrients found in foods, particularly vegetables, which have a substantial protective effect emerging from the earth, maybe conceal the presence of a few diseases (Knekt *et al.*,2002). Food consumption, particularly natural foods and green vegetables have been linked to reducing the threat and unfeeling development (Gonçalves *et al.*, 2004). In humans, cell reinforcements and the location of oxidants are stable due to equalization in regular food breakdown, which is essential for protecting the majority of favourable conditions. (Cimen, 2008).

Advanced food technology study has found that high levels of ROS can cause various diseases in humans and destroy cells, tissues, and flesh, which can be addressed with natural products such as antioxidants and natural products (Devasagayam *et al.*, 2004). Raphanus Sativus L., usually known as radish, is a widely produced Cruciferous family crop widely distributed and consumed around the world. Although the roots are the most well-known component of the radish, other sections such as the stem and leaves are also eaten by some populations as vegetables. The nutritional value of radish stems from its beneficial ingredients, including high-fibre, low-fat content, and various critical vitamins and minerals (Noman, O.M. et al.,2021). The root of the radish is the most edible component. The consumption of leaves and sprouts, on the other hand, is increasing. Salads containing the root are standard, but it can also be boiled or salted with other vegetables (Gamba, M. et al.,2021). It protects against the detrimental effects of free radicals as an antioxidant; on the other side, it helps control blood pressure and diabetes and effectively prevents colds, coughs, jaundice, asthma, constipation, and ageing. Radish (Raphanus Sativus L.) strengthens the immune system, alleviates allergies, and reduces the risk of cardiac problems, including various malignant growths.

Exogenous and endogenous sources have caused extraordinary chemical changes in natural sources that produce free radicals such as reactive nitrogen species and reactive oxygen, which have formed part of the body's defence mechanism and may protect against cancer-causing substances. Excessive production of these free radicals is hazardous since it causes humans' immune systems and other physiological systems to be destroyed (Ferreira et al., 2009). Antioxidants are prepared by the human body to guard against reactive free radicals produced by internal and external sources.

Superoxide dismutase, glutathione peroxidase, and catalase are examples of enzymes that act as antioxidants. Antioxidant enzymes are such types of enzymes (Poljsak et al., 2013). Fruits and vegetables have undeniably positive health and dietary consequences due to their ability to produce well-balanced and innovative foods, as noted by various researchers (Benzie, 2003). On the one hand, vegetables and fruits meet the fundamental nutritional requirements; on the other hand, they are good antioxidants. This antioxidant strengthens the immune system, lowers ageing signs and symptoms, energies the body, and improves walking ability (Siegrist et al., 2015).

Materials and Methods

Chemicals required

The chemicals and solvents utilized were of the highest purity and analytical quality. Methanol, Ethanol, and Deionized Water were utilized as solvents. The most common chemicals utilized were DPPH (2,2-diphnylpicrylhydrazyl),ascorbic acid (Sigma Aldrich Company), and hydrogen peroxide (H_2O_2).

Sample Collection

Three different areas were chosen for radish (Raphanus Sativus L) samples(Table: 1). During the winter seasonby following Association of Official Analytical Chemists(AOAC) international scientific method. The randomly collectedfresh samples of uprooted radish along with branches in triplicate were filled in polyethylene baggage from each specified garden.The samples were immediately transferred and stored in the laboratoryof institute of chemistry, Shah Abdul Latif University Khairpur for further analysis.

Table: 1

Radish samples were collected from various localities in Khairpur district

S. No	Code	Area	UC	Comments	Sample
1	A-01	Village Dirghpur	Wada Machhyoon	Pulp	vvv
2	A-02	Village Sadar-je-Bhatyoon	Bhatyoon	Pulp	vvv
3	A-03	Village Haji Gul Mohannad	Miahar Ali	Pulp	vvv

Sample Preparation

Radishes were carefully picked from various regions of Khairpur Mir, rinsed with tap water and then distilled water, dried, and stored in plastic bags in the refrigeratorat 3°C to 5°C.After 3 to 4 hours of adequate drying, the physical parameters were assessed using a Vernier Caliper.With the help of a pH meter, the radish juice was removed and its pH was determined (Cyber Scan pH 500 ptc. Ltd Singapore). For moisture removal and chemical analysis, all of the collected samples were homogenized and heated up to 105°C on a hot plate for one hour.

Radish Water Contents

Following the AOAC standard method, the samples were dried at room temperature before being heated in the oven at 105°C for one hour (Horwitz, 1975). The water content of chosen samples was calculated using the procedure below

$$\boxed{Mc = (Mo - Md \text{ x } 100)/(Mo)}$$

Preparation of Extract

For the purpose of extract preparation,in a round bottom flask (distillation flask), 1 gram of pulp from each sample was placed, followed by 50ml of each solvent i.e. water, methanol, water+methanolwere added to flask separately (Table: 2), the mixture was continuouslystirrer for one an hour, than cooled and dried.

Table: 2

Extract preparation in different solvents

Areas		Solvents	Total extracts
A-01	Pulp	Water	03 Pulp
		Methanol	+
		Water: Methanol	
A-02	Pulp	Water	03 pulp
		Methanol	+
		Water: Methanol	
A-03	Pulp	Water	
		Methanol	03 pulp
		Water: Methanol	=
			09 Extracts

Scavenging of free radicals

The following steps were used to determine the free radical-scavenging activity of radish (Raphanus Sativus L.) pulp samples.

DPPH Assay

As stated by the accompanying condition, the count was completed by percent inhibition: % Inhibition = $(A_b – A_s) \times 100 / A_b$

The absorbance of the emptiness is $A_b$whereas the absorbance of the sample isA_s.

DPPH (2,2-diphenol-1-picrayahydrazal) solution preparation

In an estimating carafe, a 167 M arrangement of DPPH was set up in 30 mL of methanol covered by aluminum foil.

Preparation of Antioxidant Activity Samples

1g of each homogenized sample was used to determine the radish's antioxidant activity. Then 1ml of several solvents, including water, methanol, and water + methanol, were added. After shaking, the samples were allowed to rest for around 30 minutes at room temperature. The blank sample was made in the same method but without the additions.At 517 nm, the absorbance was measured using the Cecil CE 9500 UV-Visible twin beam spectrophotometer. The concentration was estimated using the calibration graph and ascorbic acid as a standard.

Standard Solution Preparation

0.25 of ascorbic acid were weighed precisely. The sample was then dissolved in 100mls of distilled water and transferred to the 250ml measuring flask. Utilizing a UV-Visible double beam spectrophotometer, a calibration graph was created using standard ascorbic acid solutions.

Ultraviolet–Visible Spectroscopy (UV–Visible Spectroscopy)

Spectra are linked to reflectance of assimilation spectroscopy in the range of dazzling and perceptible electromagnetic radiation. In a visible area of electromagnetic range, the electronic change of particles or molecules can be observed. (Eugen, 2013).

Ultraviolet-visible Absorption Principle

Particle electrons may be resistive to holding or holding in UV or visible places, however electrons can swallow atomic orbital energise to boost life. While a smaller hole of vitality is observed in HOMO and LUMO, a longer wavelength of brightness may be retained.

Applications

UV-Visible spectroscopy offers a variety of applications as:

The quantitative and qualitative verification of diverse compounds, such as changing metals, natural macromolecules, and exceonjugated natural mixtures. Solids, like gases, can be studied using spectroscopy, which is usually done in an arrangement framework.

RESULTS AND DISCUSSION

Physical Characteristics

Radishes pulp physical characteristics were measured. The longest radish length was determined to be (44.09 cm) in area – (03), while the shortest length was calculated to be (27.01cm) in area-(02). The largest diameter of (5.01cm) was measured in area (03), while the smallest diameter of (3.01cm) was calculated in area (01).Most noteworthy load of crisp radish was observed in the samples of area – (01) as (132.2 g), whereas least mass of (111.3g) was found in the samples of area-(02).The samples from area –(01) had the highest load of crisp radish at (132.2g), while the samples from area 02 had the lowest mass at (111.3g). The most extreme load of dried radish was assessed to be (12.6g) from area –(01), with a basal load of (7.6g) from area-(03). The larger pH, moisture contents, and temperature values were determined to be 6.1, 90.8 percent, and temperature22.5^{0}C individually from three distinct districts in the Khairpurdistrict (Table: 3)

Table: 3

Radishes pulp physical properties

Areas	Color	Length (cm)	Diameter (cm)	First wt (g)	Dry wt (g)	M.C %	Pulp %	pH	Temp.
1	White	34.09	3.01	132.2	12.6	90.8	9.2	5.7	22.4
2	White	27.01	5.0	111.3	10.4	91.3	8.7	5.8	22.5
3	White	44.09	5.01	127.2	7.6	90.2	9.8	6.1	22.4

Table: 4

Radishes pulp statistical analysis

Statistics	Length (cm)	Diameter (cm)	First wt (g)	Dry wt (g)	Mc %	Pulp %	pH	Temp.
Mean	35.063	4.34	123.566	10.2	90.766	9.233	5.866	22.433
Medium	34.09	5.0	127	10.4	90.8	9.2	5.8	22.4
Range	17.08	2	20.9	5	1.1	1.1	0.4	0.1
Largest	44.09	5.01	132.2	12.6	91.3	9.8	6.1	22.5
Smallest	27.01	3.01	111.3	7.6	90.2	8.7	5.7	22.4
Sum	105.19	13.02	370.7	30.6	272.3	27.7	17.6	67.3
Count	3	3	3	3	3	3	3	3

Pulp's potential for free radical scavenging

The concentration of the pulp was plotted versus percentage (%) inhibition value on x-axis and y-axis respectively. The regression co-efficient was found to be approximately 0.98 in all cases representing the linearity of the method. The data shown as column graph and scatter graph below (figure 1.1 to 1.9) about three varieties of radish indicates that as the concentration of pulp increases than the antioxidant activity also increases.

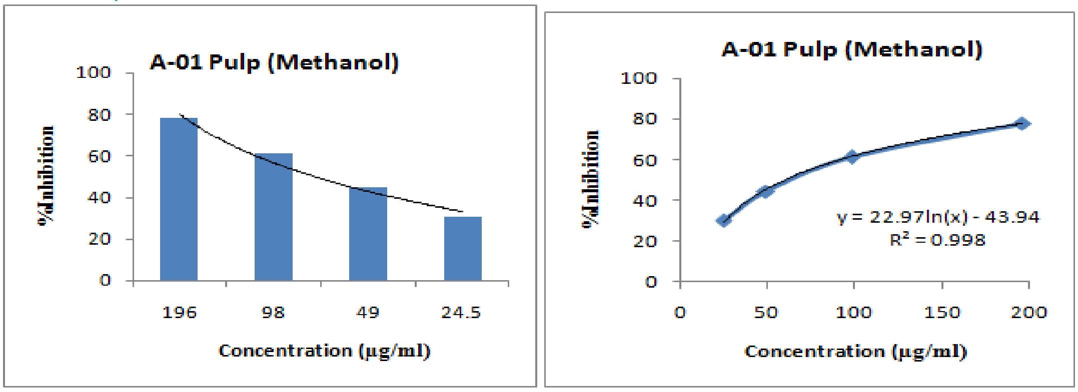

Figure.1.1 By increasing the concentration of extract A-01 in Methanol, a pattern of free radical scavenging was produced.

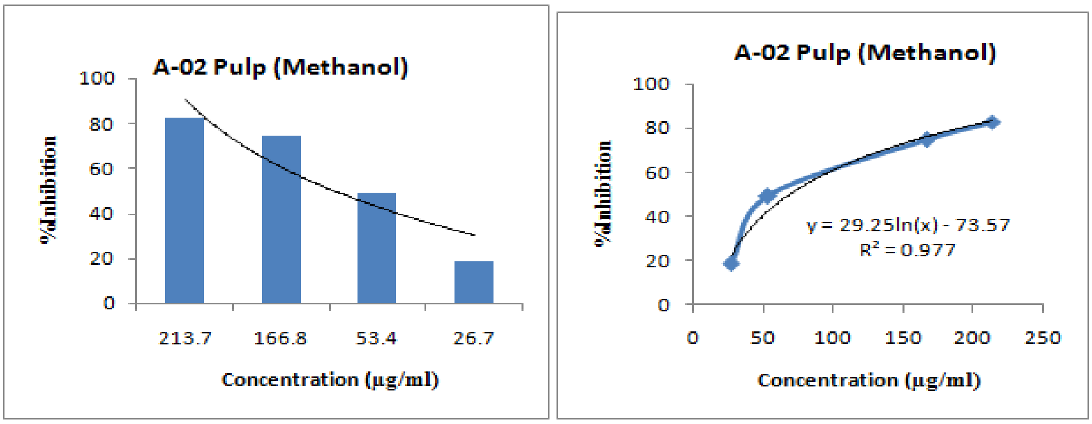

Figure.1.2 By increasing the concentration of extract A-02 in Methanol, a pattern of free radical scavenging was obtained.

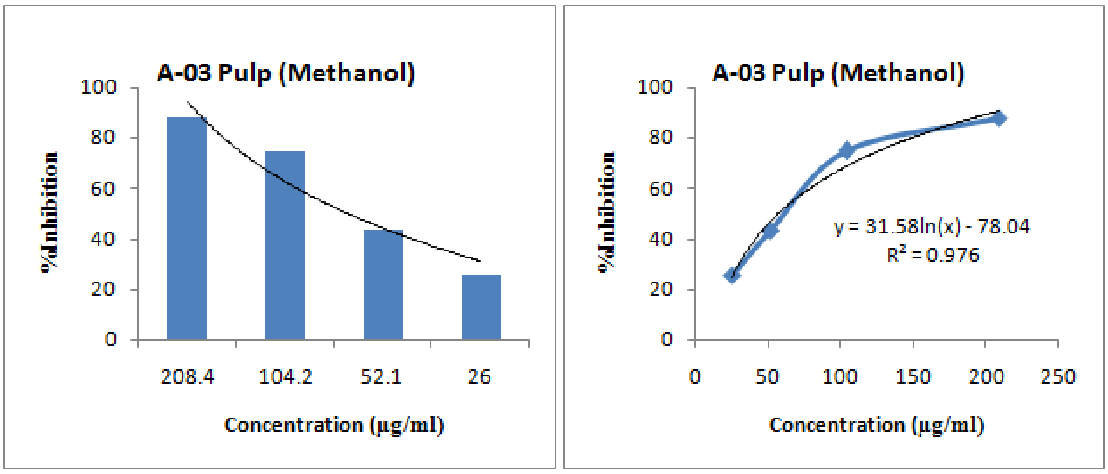

Figure.1.3 Scavenging pattern of free radicals produced by raising the content of A-03 extract in Methanol solvents

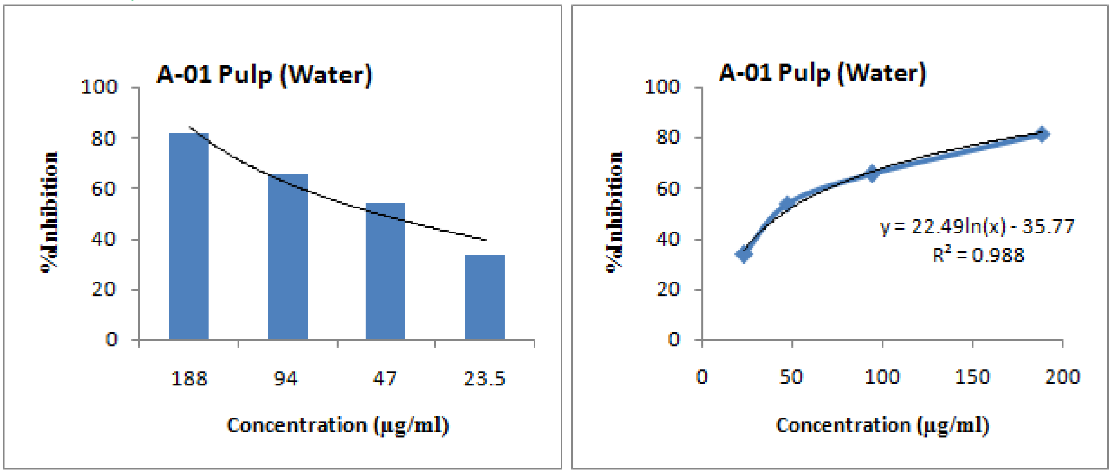

Figure.1.4 Scavenging pattern of free radicals produced byraising the content A-01 extract in water solvent.

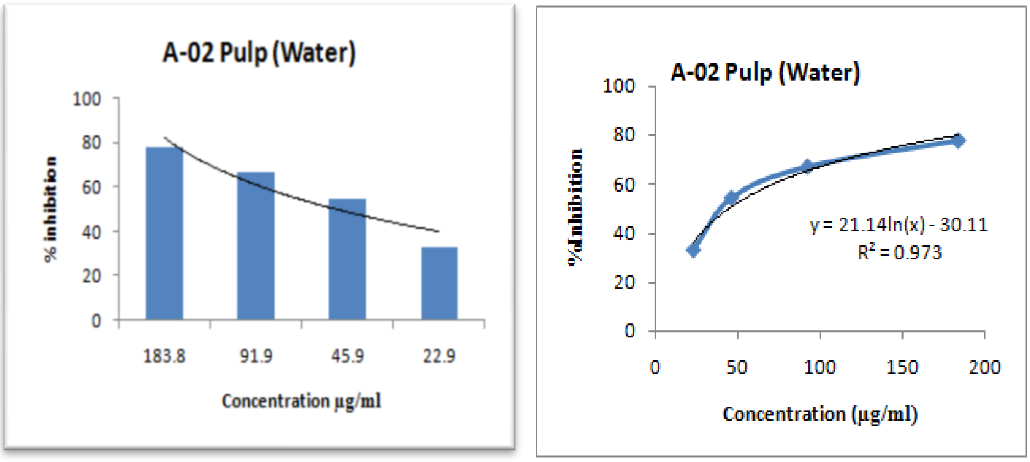

Figure.1.5 Scavenging pattern of free radicals generated by enhancing the amount of extract A-02 in water solvents.

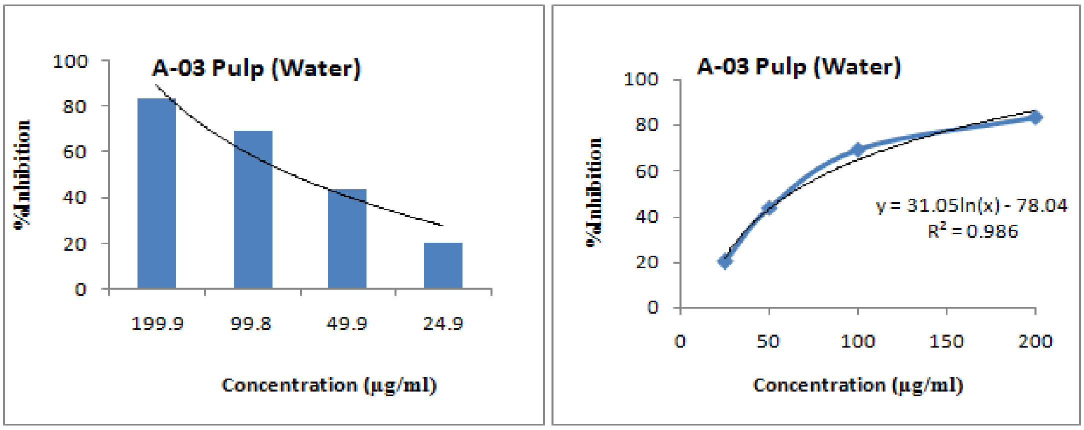

Figure.1.6 Scavenging pattern of free radicals produced by enhancing the amount of extract A-03 in water solvent.

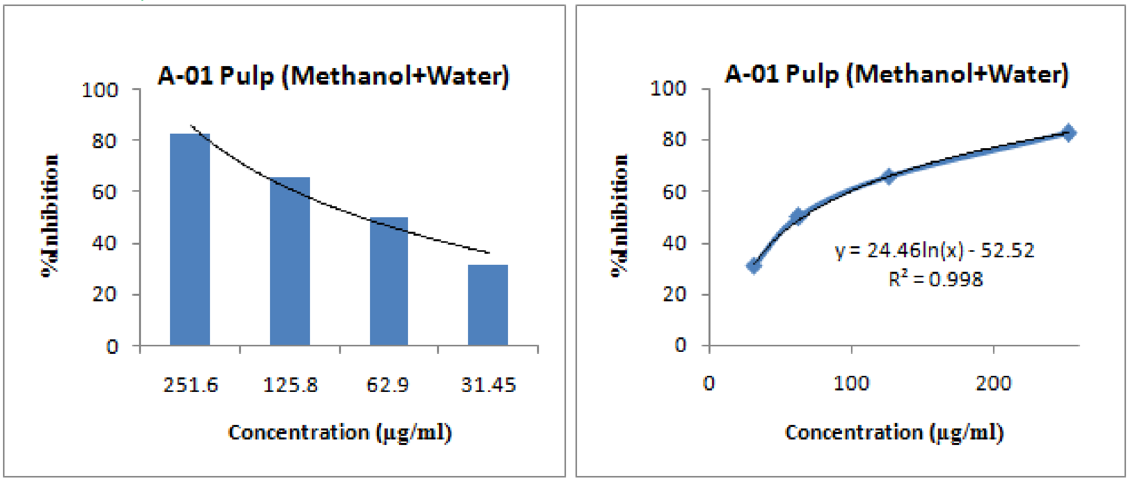

Figure.1.7 Scavenging pattern of free radicals produced by enhancing the amount of extract A-01 in Methanol+Water solvent.

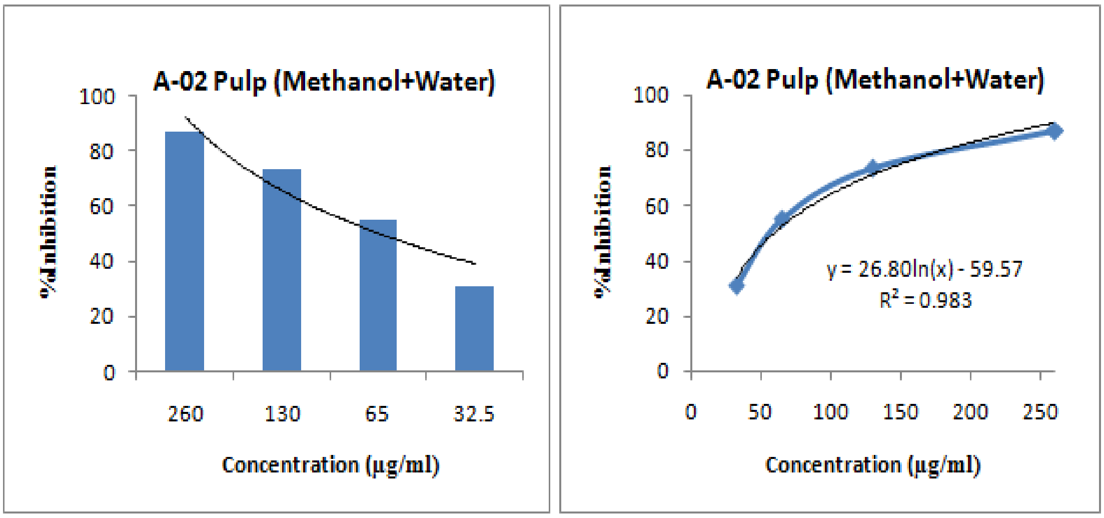

Figure.1.8 scavengingpattern of free radicals produced by enhancing the amount of extract A-02 in Methanol+Water solvent.

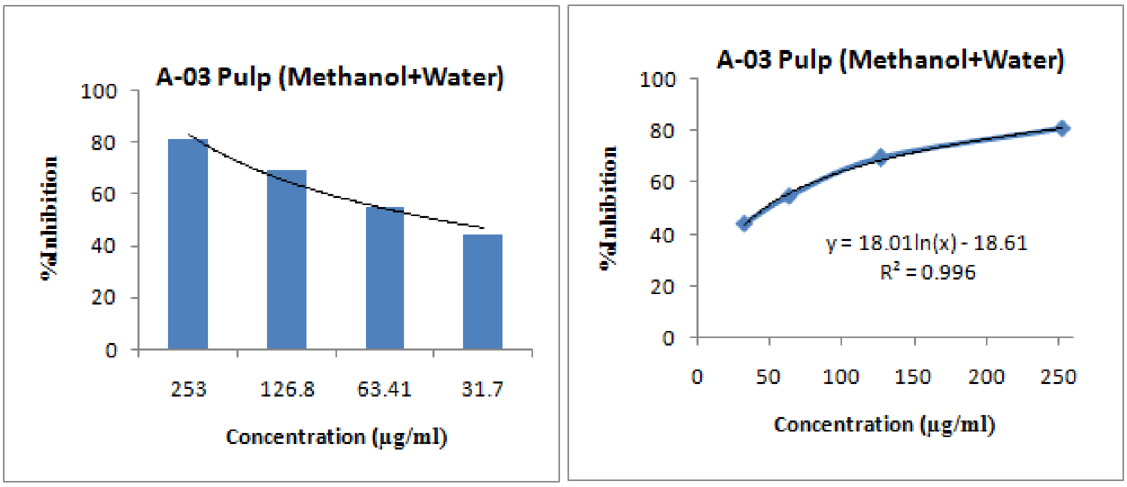

Figure.1.9 Scavenging pattern of free radicals obtained by enhancing the amount of extract A-03 in Methanol+Water solvent.

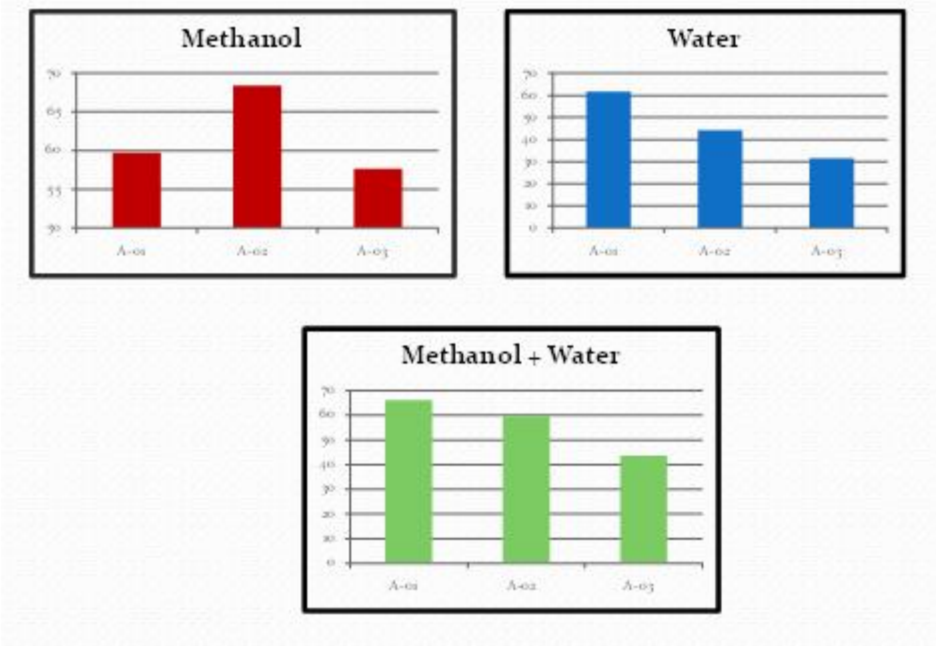

Fig.1.10 proportional IC_{50}-values of pulp

Pulp IC50-values in comparison

In three different solvents, the IC50 values of the three kinds were calculated. The low IC_{50} value in each case in methanol solvent (1.10) indicates that their antioxidant activity is maximum in methanol. Comparatively the lowest IC_{50} value in case of A-03 as 57.65µg/mlindicates its best antioxidant activity.

IC50 values of pulp against DPPH when compared to normal ascorbic acid

The IC_{50} value of three varieties and ascorbic acid (as an standard) in three solvents was observed against DPPH. The results obtained were drawn as column graph shows that though the antioxidant activity of pure ascorbic as acid is slightly better than radish but the IC_{50} values of raw radish pulp are very close to that of ascorbic acid (Figure 1.11) indicating that they are also quality antioxidants in nature.

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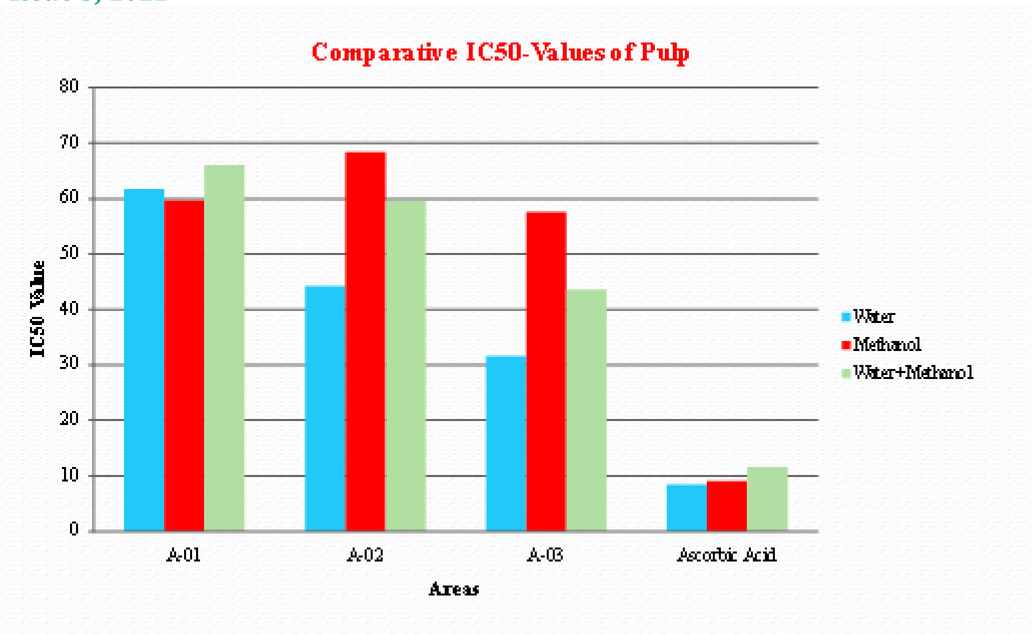

Figure.1.11 Pulp proportionate IC50 values with ascorbic acid

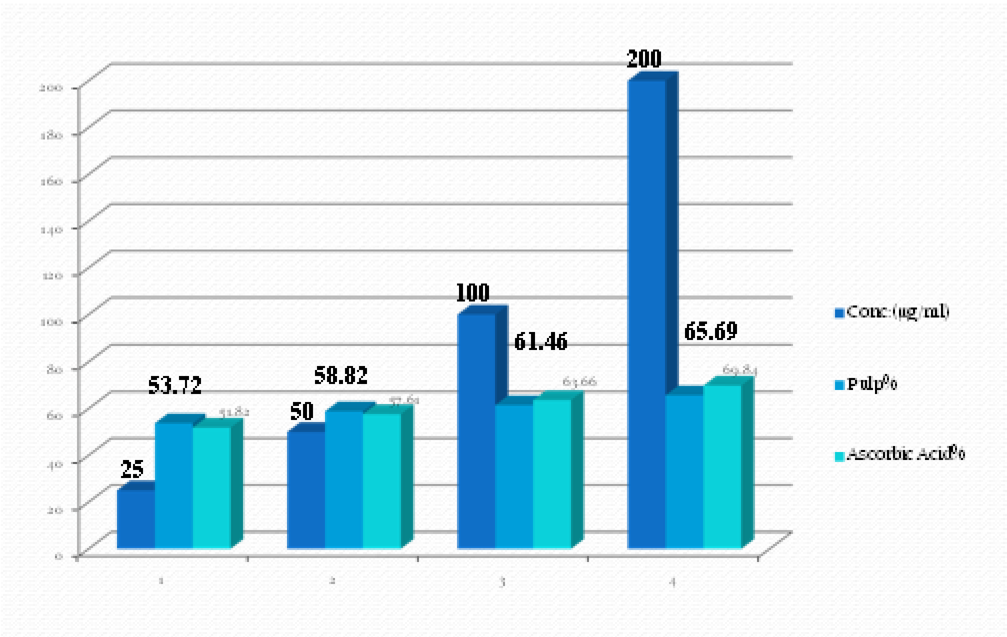

Figure.1.12 Comparison of radish extracts scavenging activity against H_2O_2 and ascorbic acid at different concentrations

Methanolic extract of radish vegetable was found to have scavenging activityat 25, 50, 100 and 200µg/ml concentrations against Ascorbic acid and H_2O_2 (Figure 1.12).Pulp shows higher activity at higher concentrations i.e. at 100 and 200 µg/mlwhile lower at lower concentration as compared with the standard (Kaur and Kapoor, 2011).

CONCLUSION

Three different varieties of radish collected from different districts of Khairpur were discovered and extracted three different solvents, methanol, water, and methanol/water (1:1) for extracting. Antioxidant activities of all the radishes were successfully found through a double beam spectrophotometer by using DPPH. It has been found that the antioxidant activity of radish was maximum in methanol compared to the water and water-methanol mixture. Radish was a great antioxidant agent as its antioxidant activity is very close to pure Ascorbic acid (standard). As the concentration of radish increases, the antioxidant activity also increases.

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