

Original Article : Open Access

Raphanus sativus L. roots: Proximate analysis and antioxidant activityRajita Beniwal, Sushila Singh[♦], Ritu Devi, Seema Sangwan*, and Parvesh Devi

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Article Info**Article history**

Received 25 January 2024

Revised 10 March 2024

Accepted 11 March 2024

Published Online 30 June 2024

KeywordsAntioxidants
Crude protein
DPPH
Flavonoids
Phenolics
Phytochemicals**Abstract**

Antioxidants are important quenchers of free radical, used to prevent and treat diseases. The presence of antioxidant in food has become an essential point of discussion. Very less data is available about the antioxidant activity of Indian food, specially when it comes to root vegetables, which is an integral part of the Indian diet. The present study aimed at extraction of phytochemicals present in *Raphanus sativus* L. root powder using different solvents and their evaluation for free radical scavenging activity using the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) method. Dried *R. sativus* powder of roots contained 13.61% of crude fiber, 11.51% crude protein; 54.20 ppm iron, 8.82 ppm copper, 19.30 ppm zinc and 107.10 ppm of manganese. The evaluation result for total sugars, non-reducing and reducing sugars were 92.95 mg/g, 90.07 mg/g, and 2.88 mg/g, respectively. Comparing the aqueous root extract to the ethanol and acetone extracts, the aqueous root extract had the highest levels of total phenolics (3.93 mg GAE/g) and flavonoids (1.06 mg CE/g). The aqueous extract was found to have the highest DPPH free radical scavenging activity, with an IC₅₀ value of 0.44 mg/ml, and it was discovered that DPPH free radical activity was concentration dependent.

1. Introduction

Humans have always used medicinal plants and herbs to treat illnesses (Moond *et al.*, 2023). The majority of the world's population about 80% resides in developing nations, where they rely on a variety of plants and the products made from them to treat a range of illnesses with minimal side effects (Shukla *et al.*, 2011; Nehra *et al.*, 2022; Devi *et al.*, 2023; Moond *et al.*, 2023). Natural antioxidants from plant sources are available as dietary supplements for use in treating and preventing oxidative stress (Krishnaiah *et al.*, 2009; Devi *et al.*, 2020; Goel *et al.*, 2022; Moond *et al.*, 2023). Different kinds of solvents are used for extracting plant metabolites and antioxidant compounds (Kumari *et al.*, 2022; Moond *et al.*, 2023). However, the solvents polarity and the chemical make-up of the extracted compounds have a significant impact on the yield and antioxidant effectiveness (Sultana *et al.*, 2009; Shabbir *et al.*, 2011; Aggarwal *et al.*, 2022; Devi *et al.*, 2023; Goel *et al.*, 2022; Moond *et al.*, 2023).

Raphanus sativus L. (Radish) belonging to Brassicaceae family, is an edible root vegetable rich in protein, carbohydrates, minerals, fat, fiber, calcium, iron, and phosphorous (Singh and Singh, 2013). *R. sativus* roots contain a variety of important bioactive substances, including fiber, polyphenols, flavonoids, carbohydrates, glucosinolates, and ascorbic acid. The butyl crotonyl isothiocyanate sulfide is the main constituent contributing to the significant odour of radish, whereas the unpleasant odour of radish is due to methyl mercaptan. *R. sativus* retains several essential properties like antioxidant, antimicrobial, antiurolithic, antifungal, and anti-

inflammatory (Siddiq and Younus, 2015). The review of the literature reveals that no systematic studies focusing on the antioxidant activity of *R. sativus* have been conducted. The goal of the current study was to estimate the phytochemicals and antioxidant activity of *R. sativus* in light of the aforementioned concern.

2. Materials and Methods**2.1 Plant material and extraction**

The Punjab safed variety of *R. sativus*. was acquired from the Department of Vegetable Science's Research Farm at the CCS Haryana Agricultural University in Hisar. The collected sample was verified by Dr. Anita, Assistant Scientist, Department of Botany and Plant Physiology, CCS HAU, by using an online platform (Tropicos and IPNI). *R. sativus* roots were skinned off, cut into pieces, and dehydrated at room temperature. They were then dried in an oven and ground into powder. Using the standard Soxhlet apparatus, 150 ml of solvents (acetone, ethanol, and water) were used to extract 10 g of powdered *R. sativus* sample (Redfern *et al.*, 2014). The residues remaining in the filter paper were again extracted for complete extraction with same solvents. The filtrates from all three extraction steps were collected and their volumes were measured. Extracts obtained in this way were also used to measure antioxidant activity and assess phytochemical content.

2.2 Chemicals

All the chemicals used to carry out experiment were of analytical grade and were obtained from Sigma-Aldrich Pvt. Ltd, an affiliate of Merck KGaA, Darmstadt, Germany; Sisco Research Laboratories Pvt Ltd Maharashtra, India. And Merck Pvt. Ltd., an affiliate of Merck KGaA, Darmstadt, Germany. DPPH and catechin were procured from Sigma-Aldrich. Na₂SO₄, Na₂CO₃, NaHCO₃, NaOH, gallic acid, sodium potassium tartrate, methanol, ethanol and acetone were purchased from SISCO research laboratories (SRL). Al₂Cl₃, NaNO₂,

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conc. HNO_3 , conc. H_2SO_4 , conc. HCl and folin-ciocalteu reagent were procured from Merck specialities.

2.3 Estimation of moisture content

The Association of Official Analytical Chemists' standard method was used to determine the moisture content (AOAC, 1990).

2.4 Estimation of crude fiber content

The improved method of Maynard was used for assessment of crude fiber (Maynard, 1970). 2 g of moisture and fat-free powder samples from the roots part of *R. sativus* were taken. Thereafter, the percentage of crude fibre content was calculated by using modified method of Maynard (1970).

2.5 Estimation of crude proteins

The conventional Micro Kjeldahl's method according to the official methods of analysis by Association of Official Agricultural Chemists (AOAC, 1990) was used for the determination of nitrogen content. The percentage of Nitrogen (N) was multiplied with factor 6.25 to assess the crude protein.

2.6 Estimation of minerals

Minerals were evaluated using the atomic absorption spectroscopy method developed by Jackson (1973) and Ruig (1986). In a conical flask, 2 g of ground *R. sativus* roots were absorbed in 15 ml of diacid mixture (4HNO_3 : 1HClO_4) by heating on a hot plate until clear white precipitate appeared at the bottom. The precipitate was dissolved in 1% HCl, sieved, and the final bulk was increased to 50 ml by adding double distilled water.

2.7 Estimation of total sugars

Total sugars were determined using the modified Dubois method (Dubois *et al.*, 1956). To keep the absorbance within calibration ranges, 1 ml of aqueous extract was diluted with distilled water (DW). Next, 2.0 ml of phenol solution (2% w/v) was added to the solution, and then 5.0 ml of concentrated sulfuric acid was added directly to the solution. A UV-Vis spectrophotometer (T60 lab india analytical) was used to measure the absorbance of the solution at 490 nm against a blank set using aqueous solvent in place of the extract after it had cooled for 30 min. The standard curve was used to calculate the extract's total sugar content, and the results were expressed in mg/g.

2.8 Estimation of reducing sugars

Reducing sugars were determined using Nelson's (1944) method, which was modified by Somogyi (1952). By diluting the 1.0 ml aqueous extract with DW, absorbance was adjusted within the calibration limit. After that, 1.0 ml of DW and 1.0 ml of alkaline copper reagent were added, and the solution was thoroughly mixed before being heated on a water bath for 20 min while wrapped in aluminium foil. After the tubes had cooled to room temperature, 1.0 ml of the arsenomolybdate reagent was added. The solution was thoroughly mixed, and the total volume was increased to 10.0 ml using DW. A UV-Vis spectrophotometer was used to measure the solution absorbance at 520 nm in comparison to a blank that was made in a comparable manner but used a different solvent in place of the extract. The amount of reducing sugars in the extracts was calculated using the standard curve, and the results were displayed in mg/g.

2.9 Estimation of non-reducing sugars

By calculating the difference between the content of total sugars and that of reducing sugars, the amount of non-reducing sugars was determined as:

Non reducing sugars = Total sugars – Reducing sugars

2.10 Estimation of total phenolics

The total phenolic content was calculated using the Folin-Ciocalteu method (Singleton and Rossi, 1965). 0.2 ml of the extract was combined with 1 mol/l of the Folin-Ciocalteu reagent. The solution was thoroughly mixed after 2.0 ml of sodium carbonate (20%, w/v) was added, and the volume was increased to 10.0 ml with DW. A UV-Vis spectrophotometer was used to measure the mixtures absorbance at 730 nm after 8 min of centrifugation at 6000 rpm for 10 min (T60 lab india analytical). As a standard, gallic acid was used to create the calibration curve. The findings were reported in milligrams of gallic acid equivalent per gram (mg GAE/g).

2.11 Estimation of flavonoids

The proportion of flavonoids in the sample was determined using a colorimetric assay with aluminium chloride (Marinova *et al.*, 2005). To 1 ml of extract, 4.0 ml of DW and 0.3 ml of NaNO_2 (5%, w/v) were added. After waiting for 5 min, 0.3 ml of AlCl_3 (10%, w/v) and 2.0 ml of 1M NaOH were added to the mixture. The volume was then increased to 10 ml using DW. After the solution had been thoroughly blended, the absorbance at 510 nm was measured using a UV-Vis spectrophotometer. Using catechin as a standard solution, a calibration curve was created, and the results were expressed as mg catechin equivalents per gram (mg CE/g).

2.12 DPPH free radical scavenging activity

The antioxidant activity of extracts was determined using the free radical scavenging method 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) (Hatano *et al.*, 1988). Extracts of *R. sativus* root powder prepared with three different solvents, acetone, ethanol, and water, were dried on a hot water bath, and the weight of dried mass was recorded after complete dehydration. To make a stock solution (50 mg/ml), a dried portion of ethanol and acetone extracts was dissolved in a suitable quantity of methanol. Because the dried aqueous extract was not soluble in pure methanol, it was dissolved in a stock solution of 50% (v/v) methanol: water. By diluting this stock solution with specific solvents, different concentrations of ethanol and acetone extract (1.0 to 50 mg/ml) and aqueous extract (0.1 to 5.0 mg/ml) were obtained (*i.e.*, used methanol for acetone and ethanol extracts and methanol: water for aqueous extracts). To determine antioxidant activity, 0.2 ml of each extract was taken and 3.0 ml of DPPH (DPPH; 0.1 mM in 100% methanol) was carefully mixed for 5 min. The stock solution of DPPH was prepared in 50% (v/v) methanol: water to determine the antioxidant activity of the aqueous extract, and the remaining method was similar. In addition, an experimental control with 0.2 ml of each solvent instead of extract was prepared. After 30 min of incubation in the dark at room temperature, the absorbance of the sample and control was measured at 517 nm using a UV-Vis double beam Spectrophotometer Model 2203 against a blank containing a specific solvent. The experiments were carried out in three replicates. The percent DPPH free radical scavenging activity on the y-axis was plotted against the concentration of extract on the x-axis in a graph. The quadratic regression equation ($y = ax^2 + bx + c$)

was then identified using Microsoft Excel, and the quadratic equation Half-maximal Inhibitory concentration (IC_{50}) was computed. The formula used to determine the percentage of DPPH scavenged (% DPPH) was:

$$\%DPPH^*_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control.

A_{sample} is the absorbance of the sample.

2.13 Statistical analysis

For statistical evaluation, the sample was collected in three copies. Using the statistical software SPSS (Statistical Package for Social Sciences) version 23, the data of proximate composition and phytochemicals were expressed as mean standard error (SE). The regression analysis of the IC_{50} values for antioxidant activity was evaluated using Microsoft Excel.

3. Results

3.1 Proximate composition, mineral content and chemical analysis

The moisture content observed in *R. sativus* roots powder was 3.40%. The crude fibre and protein content in *R. sativus* roots were found to be 13.61 and 11.51 %, respectively (Figure 1). In mineral analysis, the mineral content in *R. sativus* roots, the minerals (Fe, Mn, Zn and Cu) content was estimated and data is presented in Table 1. Minerals iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) content in experimental samples were found to be 54.20, 107.10, 19.30 and 8.82 ppm, respectively. The total sugar content, non-reducing sugars and reducing sugars in aqueous extract of *R. sativus* roots were estimated as 92.95, 2.88 and 90.07 mg/g, respectively (Table 1).

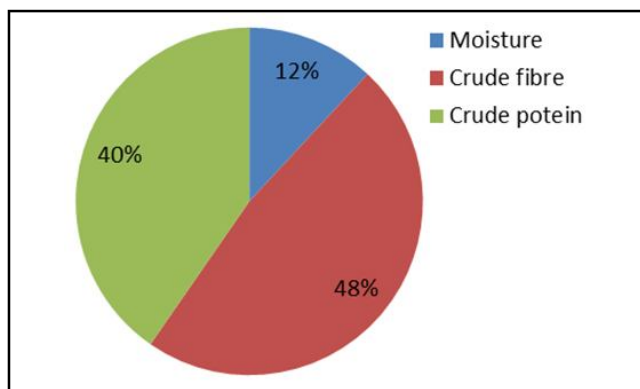


Figure 1: Proximate composition (%) of roots of *R. sativus* .

Table 1: Chemical composition of *R. sativus* root powder

| Parameters | Results |
|----------------------|-------------------|
| Fe | 54.20 ± 0.32 ppm |
| Mn | 107.10 ± 0.17 ppm |
| Zn | 19.30 ± 0.11 ppm |
| Cu | 8.82 ± 0.06 ppm |
| Total sugars | 92.95 ± 0.59 mg/g |
| Reducing sugars | 90.07 ± 0.65 mg/g |
| Non- reducing sugars | 2.88 ± 0.19 mg/g |

3.2 Effect of extracting solvents on phytochemical parameters

A standard curve using gallic acid as standard was prepared for the estimation of total phenolics content in aqueous, acetone and ethanolic extracts of radish (Punjab safed) roots (Figure 2). The regression equation obtained between absorbance (y) and amount of gallic acid (x, in mg) was:

$$y = 0.0086x + 0.0617 \quad (R^2 = 0.9989)$$

This equation showed a linear relation between absorbance and amount of gallic acid.

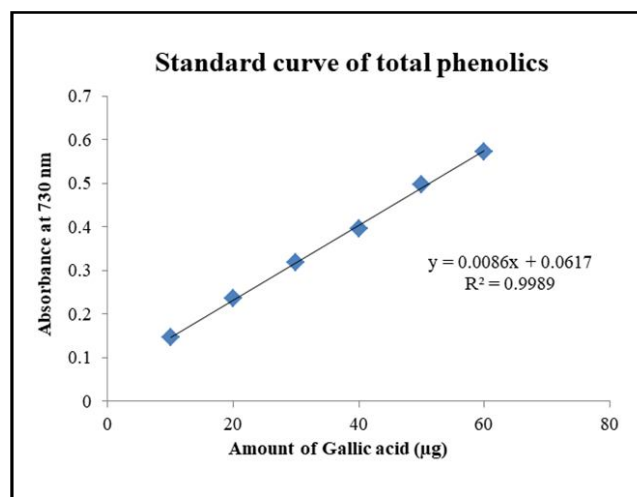


Figure 2: Standard curve of total phenolics using gallic acid as standard.

The total phenolic content in different solvent extracts of *R. sativus* roots has been presented in Table 2. Aqueous extract of *R. sativus* contains maximum total phenolic content, i.e., 3.93 milligrams of gallic acid equivalent per gram (mg GAE/g) compared to 3.70 mg GAE/g in ethanol extract and 0.61 mg GAE/g in acetone extract based on the dry weight. A standard curve using catechin as standard was prepared for the estimation of aqueous, acetone and ethanolic extracts of *R. sativus* roots (Figure 3). The following regression equation was obtained between absorbance (y) and amount of catechin (x, in mg):

$$y = 0.0033x - 0.0133 \quad (R^2 = 0.999)$$

This equation shows a linear relation between absorbance and amount of catechin.

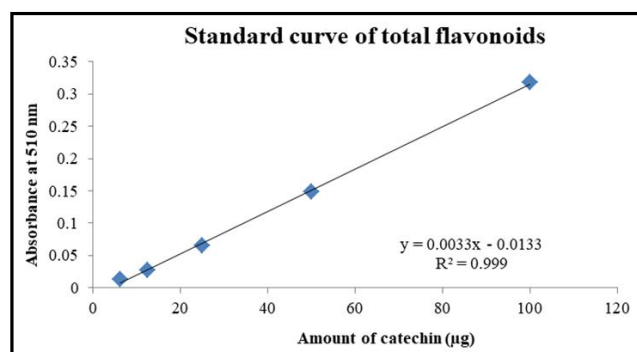


Figure 3: Standard curve of total flavonoids using catechin as standard.

Broad variation has been observed in the flavonoid content of *R. sativus* root extracts in three solvents, as shown in Table 2. Based on dry weight, the aqueous extract of *R. sativus* contained maximum

total flavonoid content, *i.e.*, 1.06 mg CE/g in comparison to 0.48 mg CE/g and 0.25 mg CE/g in ethanol and acetone extracts, respectively.

Table 2: Total phenolic and flavonoid contents of *R. sativus* root extracts prepared using different solvents

| Solvent | Total phenolic content (mg GAE/g) | Total flavonoid content (mg CE/g) |
|---------|-----------------------------------|-----------------------------------|
| Acetone | 0.61 ± 0.02 | 0.25 ± 0.00 |
| Ethanol | 3.70 ± 0.04 | 0.48 ± 0.00 |
| Water | 3.93 ± 0.01 | 1.06 ± 0.01 |

3.3 DPPH free radical scavenging activity

When one electron is removed from 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), a constant free radical with a purple colour, it transforms into a non-radical form with a yellow colour. In this study, DPPH

free radical scavenging activity (%) was found to be ranged from 1.01 to 76.25 % in aqueous extract, from 6.53 to 76.51% in ethanol extract and from 5.73 to 76.20% in acetone extract corresponding to 0.1 to 2.5 mg/ml for aqueous and 1.0 to 50.0 mg/ml for ethanol and acetone extracts, respectively (Figure 4).

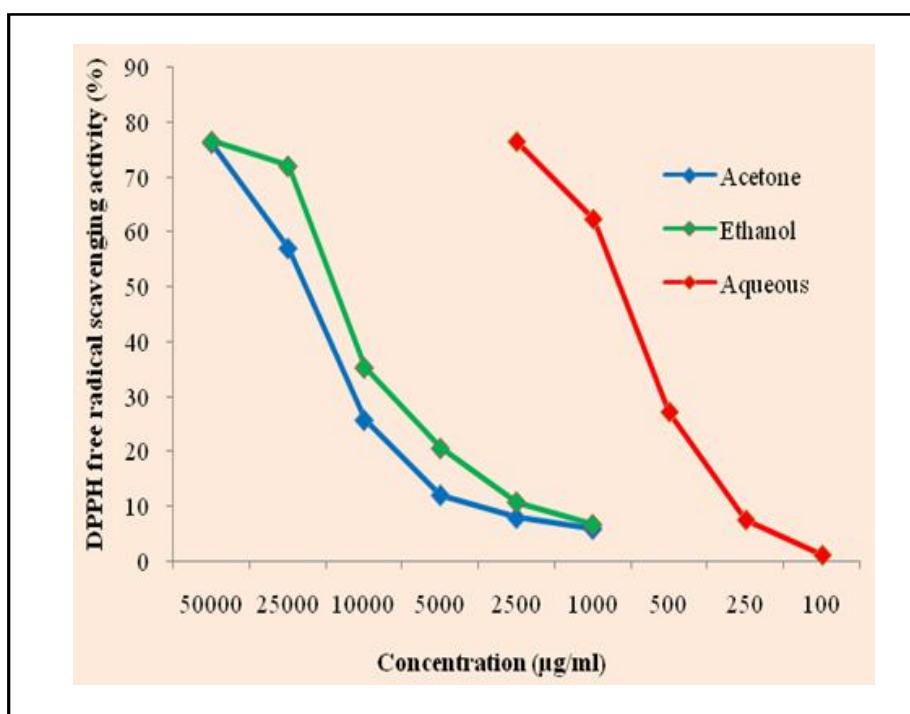


Figure 4: DPPH free radical scavenging activity (%) of acetone, ethanol and aqueous extracts of *R. sativus* roots.

The IC_{50} value of aqueous extract was lowest 0.44 mg/ml, followed by 14.67 mg/ml of ethanol extract and 21.42 mg/ml (Table 3) of

acetone extract, thereby showing that aqueous extract has highest activity followed by ethanol and acetone extracts.

Table 3: Quadratic regression equation and IC_{50} value of *R. sativus* roots

| S. No. | Parameters | Acetone extract | Ethanol extract | Aqueous extract |
|--------|-------------------------------|---|---|---|
| 1. | Quadratic regression equation | $y = -0.026x^2 + 2.846x + 0.966$ $R^2 = 0.997$ | $y = -0.051x^2 + 4.069x + 1.284$ $R^2 = 0.998$ | $y = -24.26x^2 + 96.10x - 12.04$ $R^2 = 0.991$ |
| 2. | IC_{50} (mg/ml) | 21.42 | 14.67 | 0.44 |

4. Discussion

Natural antioxidants and their connection to health advantages have received a lot of attention recently. Free radicals can be produced by oxidation reactions, which the immune system must eliminate because

they typically attack cell membranes and cause cell damage. Antioxidants improve the shelf life and quality of food products, reduce oxidative damage in foods, and protect biological systems by delaying or preventing the oxidation process brought on by reactive

oxidation species. Since they have such a high potential for promoting health, are so safe, and are so widely accepted by consumers, there has been a significant increase in interest in discovering naturally occurring antioxidants that can be used in foods recently (Gorinstein *et al.*, 2003).

The powdered *R. sativus* roots contained the following amounts of crude protein (11.51%), crude fibre (13.61%), and moisture (3.40%). Other researchers have reported similar outcomes; one study (Nhu *et al.*, 2014) found that *R. sativus* root powder has a moisture content of 5.60%. The results for crude fibre are comparable to those from the earlier study, which found 0.8% (fresh weight basis) fibre in *R. sativus* roots (Singh and Singh, 2013). *R. sativus* roots had a crude protein content of 0.7 g/100 g (dry weight basis). Similar results have been reported by other researchers, and a report demonstrates the moisture (Singh and Singh, 2013).

In the current study, total sugars, reducing sugar and non-reducing sugars were found to be 92.95, 90.07 and 2.88 mg/g, respectively. Our findings are consistent with the earlier study, which found that the roots of *R. sativus* had a total sugar content of 45.3 mg/g (Prasad *et al.*, 2015). According to a study of reducing sugars, the content has been reported as 84.95 mg/g in roots of *R. sativus* (Nhu *et al.*, 2014). Results of other researchers showed that non-reducing sugar content varies between 0.9 to 3.0 mg/g in roots of *R. sativus* which is in accordance with our result (Jahan *et al.*, 2011).

The amounts of total phenolics in *R. sativus* root extracts in acetone, ethanol, and water were found to be 0.61, 3.70, and 3.93 mg/g, respectively. This result is in accordance with the findings of other researchers who have reported the presence of 4-5 mg GAE/g of total phenolics in roots extract of *R. sativus* (Bors *et al.*, 2015). According to the study conducted on Rang Chuet (*Thunbergia laurifolia* Lindl.), water extract contains the maximum phenolics content followed by ethanol and acetone extracts (Oonsivilai *et al.*, 2008).

Total flavonoids content varied from 0.25, 0.48 and 1.06 mg/g in acetone, ethanol and water extract, respectively. The findings are similar as reported by other researchers, *i.e.*, 0.45 mg QE/g in acetone roots extract of *R. sativus* (Saikia and Mahanta, 2013). According to a study conducted on *R. sativus* roots, the total flavonoid content was found to be 0.33- 0.51 in different extracts (Jakmatakul *et al.*, 2009).

In this study, the acetone, ethanol, and aqueous extracts of *R. sativus* root showed significantly different DPPH free radical scavenging activity (%) that increased with increasing concentration levels as shown in Table 3. It ranged from 1.01 to 76.25% in aqueous extract, from 6.53 to 76.51% in ethanol extract and from 5.73 to 76.20% in acetone extracts corresponding to 0.1 to 2.5 mg/ml for aqueous and 1.0 to 50.0 mg/ml for ethanol and acetone extracts, respectively.

Aqueous extract had the lowest IC₅₀ value (0.44 mg/ml), compared to ethanol extract 14.67 mg/ml and acetone extract 21.42 mg/ml. The results are consistent with research done on Rang Chuet (*Thunbergia laurifolia* Lindl.), where aqueous extract showed the greatest ability to scavenge DPPH free radicals (IC₅₀ = 0.13 mg GAE/ml), while ethanol and acetone extracts were found to have IC₅₀ values of 0.26 and 0.61 mg GAE/ml, respectively (Oonsivilai *et al.*, 2008). *R. sativus* root aqueous extract had antioxidant activity ranging from 13.71 to 58.38%, with an IC₅₀ value of 0.17 mg/ml (Agarwal and Varma, 2014).

5. Conclusion

Outcomes of current experimental findings demonstrate that the solvent played a significant role in the extraction of phytochemicals and their antioxidant activity. The aqueous extract fraction of *R. sativus* contained the highest content of total phenolics and flavonoids and also showed maximum antioxidant activity when compared to ethanol and acetone extracts. Radish is a good source of crude fiber and minerals (Fe, Cu, Zn, Mn). The result of the present study will also serve as reference material in the preparation of herbal material as well as in pharmaceutical industries.

Acknowledgements

The authors gratefully acknowledge the Department of Chemistry at Chaudhary Charan Singh Haryana Agricultural University, Hisar, for providing the necessary resources.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation

Rajita Beniwal, Sushila Singh, Ritu Devi, Seema Sangwan, and Parvesh Devi (2024). *Raphanus sativus* L. roots: Proximate analysis and antioxidant activity. Ann. Phytomed., **13**(1):1270-1275. <http://dx.doi.org/10.54085/ap.2024.13.1.138>.