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PHYTOCHEMICAL PROFILING AND NUTRITIONAL CHARACTERIZATION OF *RAPHANUS SATIVUS* ROOTS

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ABSTRACT
Radish (*Raphanus sativus*) is the most consumed vegetable worldwide due to its potential health benefits. The current study aimed to explore the nutritional composition and therapeutic potential of Raphanus sativus roots. The proximate analysis of *Raphanus sativus* roots indicated that 90.23±0.92%, 0.65±0.03, 0.10±0.005%, 0.11±0.01%, 0.52±0.02%, 8.39±0.96% moisture, crude protein, crude fat, crude fiber, ash and NFE, respectively. *Raphanus sativus* roots were found to be rich in potassium (231.31±0.58 mg/100g), sodium (50.86±0.16 mg/100g), calcium (38.55±0.05 mg/100g) and also have a good amount of magnesium, iron and zinc. The phytochemical and antioxidant properties of *Raphanus sativus* roots by using different solvents (Aqueous and ethanol) were measured. Furthermore, phytochemical analyses expressed that the ethanolic extract of Raphanus sativus showed a significantly ($p<0.05$) higher ratio of Total phenolic content (TPC), Total flavonoid content (TFC) and antioxidant capacity than the aqueous extract.

Keywords: *Raphanus sativus* roots, Nutritional composition, Phytochemical analysis, Antioxidants

1. INTRODUCTION
Raphanus sativus belongs to the Brassicaceae family; it has various names as it is known as Muli and Mooli in Punjabi/Hindi and Radish in English (Chen *et al.,* 2023). It is cultivated and consumed in the entire world (Ali *et al.,* 2023). Antioxidants, phytochemicals, polyphenols, secondary metabolites, polysaccharides, minerals, and vitamins in leaves and roots make it a therapeutic plant (Zappia *et al.,* 2023; Shin *et al.,* 2015). Moreover, the bioactive compounds and antioxidant activity of *Raphanus sativus* leaves are four times higher as compared to roots (Goyeneche *et al.,* 2015). It contains numerous efficient secondary metabolites that act both pharmacologically and therapeutically (Akram et al., 2015). There are several varieties of Raphanus sativus e.g., black radish, red radish, and white radish (Elamin, 2015). *Raphanus sativus* is among the most popular root vegetables that are grown in temperate, tropical, and sub-tropical regions worldwide. *Raphanus sativus* is cultivated and consumed in the entire world. However, it is not commonly used in a few populations, but it is considered as a part of the human diet. *Raphanus sativus* contains mustard oil which gives it a pungent taste and smell, also considered as an appetizer. Raphanus sativus can also be eaten or cooked as a vegetable and contains vitamins such as vitamin A and vitamin C and minerals like calcium, phosphorus, potassium etc. *Raphanus sativus is* also blessed with refreshing and diuretic properties (Satish, 2016). *Raphanus sativus* is considered to have a broad variety of secondary metabolite such as phenolic, coumarins,

antioxidant enzymes, glucosinolates, alkaloids, flavonoids (anthocyanin), carotenoids, terpenes, anthocyanins and isothiocyanates, that are linked to its antioxidant properties (Shin et al., 2015; Kim et al., 2015; Beevi et al., 2012). The roots of *Raphanus sativus* contain several phenolic acids such as p-coumaric acid, transferulic acid, vanillic acid and caffeic acid. However, leaves of the *Raphanus sativus* contain caffeic acid, epicatechin, transsinapic acid, p-coumaric acid, transferulic acid and tyrosol in free form while, transferulic acid, vanillic acid and p-coumaric acid are bounded. In roots, the free form accounts for 32% while in leaves, it accounts for almost 55% of total phenolic compounds. Free hydroxyl groups present in phenolic substances are responsible for the antioxidant activity (Zhang et al., 2014). Vanillic acid and pyrogallol acids are the most abundant bound or free phenolic substances present in the roots.

Phytochemicals are plant based chemical compounds that work as an antioxidant and scavenge reactive oxygen species, resulting in reduction of oxidative stress. Numerous amounts of phytochemicals can be identified in plants and a single plant possessing different medicinal health properties (Keyata et al., 2021). Phytochemical evaluation showed that the *Raphanus sativus* contains saponins, phlobatannins, carbohydrates, steroids, alkaloids, terpenoids, chalcones, tannins, flavonoids, anthraquinones, reducing sugars, phytosterols, amino-acids and cardiac glycosides (Umamaheswari et al., 2021). *Raphanus sativus* extracts possess high flavonoid and phenolic content. These compounds exhibit antioxidant properties which act as hepatoprotective agents either indirectly by enhancing the tissue antioxidant enzyme activity or directly by interacting with toxic (Kasote et al., 2015). Depending on the abovementioned evidence, this research work is designed to evaluate the phytochemical and nutritional characteristics of *Raphanus sativus* roots.

2. MATERIALS AND METHODS

2.1 Preparation of raw material

Raphanus sativus roots were obtained from the local market in Faisalabad. After cleaning and washing, they were screened for impurities. Afterward, the *Raphanus sativus* roots were dried in a Hot Air Oven at 55-60ºC for 16 to 18 hours (Magied et al., 2016).

2.2 Proximate analysis of Raphanus sativus roots

Proximate analyses, encompassing moisture, ash, crude protein, crude fat, crude fiber, and nitrogen-free extract (NFE) of *Raphanus sativus* roots, were determined according to the protocol described by AOAC (2016).

2.3 Minerals analysis of Raphanus sativus roots

The levels of available minerals in *Raphanus sativus* roots were determined following the procedures outlined in AOAC (2016). A Flame Photometer was utilized for sodium (Na), calcium (Ca), and potassium (K), while an Atomic Absorption Spectrophotometer was employed to analyze magnesium (Mg), zinc (Zn), iron (Fe), and manganese (Mn) in *Raphanus sativus* roots.

2.4 Total Phenolic Contents

Total phenolic contents of Raphanus sativus root extracts were estimated by using the standard Folin-Ciocalteu method as described by Goyeneche et al. (2015). In this regard, 0.5 mL of leaves extract and 0.5 N Folin-Ciocalteu reagent, and 2 mL of 20% sodium carbonate solution were added and mixed well. The mixture was incubated at room temperature (25 ℃) for 30 minutes, afterward taken in a test tube and mixed well. Later, the absorbance was measured by using a spectrophotometer at 765 nm and the total phenolic contents were expressed as Gallic Acid Equivalent (GEA) in mg/g extract.

2.5 Total Flavonoid Contents

Total flavonoid contents from *Raphanus sativus* root extracts were obtained according to the procedures of Goyeneche et al. (2015) with some modifications. Purposely, 0.1 mL leaves extract was added to 1 mL of 10% AlCl3 prepared in methanol. Afterward, the solution was mixed thoroughly and allowed to incubate for 30 minutes. Finally, the absorbance of the mixture was taken at 510 nm

by using a spectrophotometer. The concentration of flavonoids in *Raphanus sativus* leaves extract was measured and expressed as mg quercetin equivalent (QE)/g of the extract.

2.6 Antioxidant Activity by DPPH Assay

Antioxidant activity of *Raphanus sativus* roots extract was performed by using 2, 2-diphenyl-picrylhydrazyl (DPPH) according to the protocol described by Beevi et al. (2012). In this regard, 1 mL of extract was mixed with 1 mL of 1 mM DPPH solution and methanol. After that, shake the mixture for 1 min and incubate at room temperature (25 ℃) for 30 min. Later, the absorbance of the sample was recorded at 517 nm.

2.7 Ferric Reducing Antioxidant Power Assay

Ferric reducing antioxidant power (FRAP) of the extract was assessed by using the protocol of Beevi et al. (2012). For preparing the FRAP reagent, 10 mM of TPTZ (2.5 mL), 0.1 M acetate buffer (25 mL), and 20 mM ferric chloride (2.5 mL) were mixed thoroughly and followed by incubation for 10 minutes. Hence, extract/standard (50 µL) was added to FRAP reagent (1 µL) and triple dH2O (100 µL). Later, the absorbance of the samples/blank was noted at 593 nm via UV/Visible spectrophotometer. Using ferrous sulfate (0-500 µmol per mL), a calibration curve was obtained. The results have been reported as µmol ferrous sulfate per gram of extract (µmol FeSO4/g).

3. RESULTS

3.1 Proximate analysis

The proximate analysis of *Raphanus sativus* roots revealed the moisture content, crude protein, crude fat, crude fiber, total ash, and nitrogen-free extract as 90.23±0.92, 0.65±0.03, 0.10±0.005, 0.11±0.01, 0.52±0.02 and 8.39±0.96%, correspondingly as shown in Table 1.

Table 1. Proximate composition of *Raphanus sativus* roots

Component	Means
Moisture	90.23±0.92
Crude Protein	0.65±0.03
Crude Fat	0.10±0.005
Fiber	0.11±0.01
Ash	0.52±0.02
NFE	8.39±0.96

3.2 Minerals analysis of *Raphanus sativus* roots

Minerals are inorganic compounds required in minute amounts for the proper functioning of the body. The *Raphanus sativus* roots are excellent source of minerals such as potassium (231.31±0.58 mg/100g), sodium (50.86±0.16) calcium (38.55±0.05 mg/100g), magnesium (12.21±0.01 mg/100g), iron (0.67±0.01 and zinc (0.31±0.005) respectively as shown in (Table 2).

Table 2. Minerals analysis of *Raphanus sativus* roots

Minerals	Means (mg/100g)
Potassium	231.31±0.58
Sodium	50.86±0.16
Calcium	38.55±0.05
Magnesium	12.21±0.01
Iron	0.67±0.01
Zinc	0.31±0.005

3.3 Phytochemical Analyses

The phytochemical properties of aqueous and ethanolic extracts were determined to check the antioxidant potential of *Raphanus sativus* roots.

3.3.1 Total phenolic contents

The total phenolic contents were higher in the ethanolic extract of *Raphanus sativus* roots (80.85±2.0 mg GAE/g) as compared to the aqueous extract (33.32±1.33 mg GAE/g) as presented in Table 3.

3.3.2 Total flavonoid contents

The total flavonoid contents were found higher in the ethanolic extract of *Raphanus sativus* roots (26.22±2.13 mg QE/g) as compared to the aqueous extract (11.65±1.58 mg QE/g).

3.3.3 Antioxidant capacity by DDPH

DDPH free radical scavenging method is used because of its stable radical antioxidant reaction. The maximum value for DPPH activity was determined in the ethanolic extract of roots (69.00±2%) while in the aqueous extract was (38.00±1.52%). The recorded DPPH values are as reported in the results Table 3.

3.3.4 Ferric Reducing Antioxidant Power

Ferric reducing antioxidant power assay measures the ferric-to-ferrous reduction capability of an antioxidant. The highest activity was found in the ethanolic extract of roots (1.62±0.06 mM Fe2+/g) while the lowest radical scavenging activity was observed in the aqueous extract (1.32±0.05 mM Fe2+/g), respectively.

Table 3. Mean values for phytochemicals and antioxidant activity of *Raphanus sativus* roots

Antioxidant activities of *Raphanus sativus* roots	**Aqueous**	**Ethanolic**
TPC (mg GAE/g)	33.32±1.33	80.85±2.0
TFC (mg QE/g)	11.65±1.58	26.22±2.13
DPPH (%)	38.00±1.52	69.00±2
FRAP (mM Fe^{2+}/g)	1.32±0.05	1.62±0.06

4. DISCUSSION

The current study demonstrates the proximate, mineral composition of *Raphanus sativus* roots. Proximate analyses were done to quantify the macro-molecules, i.e., moisture, ash, crude fat, protein, fiber and nitrogen free extract (NFE) in *Raphanus sativus* roots. The food commodity and its composition greatly impact the quality of the final product, therefore, compositional analysis significantly contributes to decide the important ingredients in the product recipe. The proximate analyses of *Raphanus sativus* roots showed the moisture, crude protein, crude fat, crude fiber, ash content and NFE as 90.23±0.92 , 0.65±0.03, 0.10±0.005, 0.11±0.01, 0.52±0.02 and 8.39±0.96 and 11.22±0.27%, respectively (Table1.).The moisture content of Raphanus sativus roots and leaves was evaluated on wet weight basis and after that, the dry powder was used for further analysis.

The trend of this proximate composition is in-line with the findings of Magied et al. (2016). Accordingly, *Raphanus sativus* roots and leaves contain moisture content of 90.23±0.92 g/100g. In another investigation,

Goyeneche et al. (2015) determined the proximate composition of red radish roots and leaves. According to the research on red radish roots moisture content, the stated values were 95.24±0.29 g/100g. Janjua and Shahid (2013) also determined the moisture content of *Raphanus sativus* root peel up to 7% on a dry weight basis. Proteins are nitrogenous combinations composed of amino acids, the main building blocks of our body tissues like nails, hair, antibodies and most importantly muscles etc. The amount of protein in the roots of *Raphanus sativus*, 0.65±0.03%.

Different research has shown the natural composition of *Raphanus sativus* roots. Magied et al. (2016) carried out a study in which they reported the crude protein percentage of *Raphanus sativus* roots as 14.9±0.25 g per 100g, respectively on dry weight basis. While Goyeneche et al. (2015) found protein content in roots 0.57±0.09 g/100g, respectively. The fat content of *Raphanus sativus* roots (0.10±0.005 %) of current investigation was comparable to the finding of

Goyeneche et al. (2015), who reported crude fat in *Raphanus sativus* roots as 0.07±0.01 g per 100g, respectively. Later, Magied et al. (2016) investigated the crude fat content in Raphanus sativus roots (2.11±0.21 g/100g),

However, it was on dry weight basis. The amount of crude fiber in *Raphanus sativus* roots and leaves in the current study was 0.11±0.01% and 0.23±0.05%, respectively. According to Goyeneche et al. (2015), the crude fiber in roots was 0.32±0.07 g/100g, correspondingly. Later, Magied et al. (2016) determined that the crude fiber in *Raphanus sativus* roots as 12.9±0.35 g/100g, respectively on dry weight basis. Ash is an inorganic material which remains after the complete burning of a sample at high temperature. The present research work indicates that the ash content of *Raphanus sativus* roots was 0.52±0.02% and 1.41±0.03%, respectively. These results are comparable to the investigation of Goyeneche et al. (2015), who reported the ash content in *Raphanus sativus* roots as 0.77±0.07 g per 100g, respectively. Magied et al. (2016) determined that the ash content of *Raphanus sativus* roots 13.2±0.25 g per 100g on dry weight basis. Nitrogen free extract (NFE) generally involves carbohydrates i.e. starches & sugars as a main component of hemicellulose in foodstuffs. Recent investigation indicates that the

NFE of *Raphanus sativus* root (8.39± 0.96%) According to Goyeneche et al. (2015), the Nitrogen free extract of red radish roots 3.03 to 4.04 g/100g. A group of peers determined NFE carbohydrates as 39.82% in *Raphanus sativus* root peel on a dry weight basis (Janjua and Shahid, 2013). Moreover, the mineral content of *Raphanus sativus* roots showed potassium (231.31±0.58 mg/100g) as a major mineral, followed by sodium (50.86±0.16 mg/100g), calcium (38.55±0.05 mg/100g), magnesium (12.21±0.01 mg/100g), iron (0.67±0.01 mg/100g) and zinc (0.31±0.005 mg/100g), respectively. Overall, results indicated that mineral content was higher in leaves than roots. Significant differences were detected in aqueous and ethanolic extracts for their phytochemical content and antioxidant capacity analyzed via TPC, TFC, DPPH, and FRAP assays. The highest phenolic content was found in ethanolic extract of roots as 113.22±2.83 and 80.85±2.0 mg GAE/g than aqueous extract of roots i.e., and 33.32±1.33 mg GAE/g. The total flavonoid content of *Raphanus sativus* roots a was found maximum in ethanolic extract (26.22±2.13 mg QE/g) and minimum amount was recorded for aqueous extract of roots (11.65±1.58 mg QE/g). The antioxidant activity based on DPPH assay was highest in ethanolic extract of *Raphanus sativus* roots as 69.00±2.00%, respectively. Similarly, maximum ferric reducing ability was observed in ethanolic extract of roots (1.62± 0.06 mM Fe 2+ /g). The little variations in the proximate composition are due to varietal, soil and environmental differences that significantly affect the structure of fruits and vegetables.

CONCLUSIONS

The current research work was performed to check the phytochemical profiling and nutritional characterization of *Raphanus sativus* roots. The proximate analysis indicates that *Raphanus sativus* roots contain a high ratio of moisture content. *Raphanus sativus* roots are good sources of potassium, calcium, sodium, magnesium and iron which indicates its importance in antioxidant effects in the human body. Additionally, awareness sessions should be conducted to explain the medicinal importance of roots against different diseases. However, more research is required to approve the safety aspects of plant-based food products before inducing them into the human diet.

***Conflict of Interest**:* The authors declare no conflict of interest.

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