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Research Article

ANTIOXIDANT ACTIVITY OF *RAPHANUS SATIVUS* L. OF JHANSI DISTRICT, UTTAR PRADESH, INDIA

Ramesh Kumar *, Roshni Patwa
Department of Biochemistry, Bundelkhand University, Jhansi, Uttar Pradesh, India
*Corresponding Author Email: ramesh_1995in@yahoo.co.in

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ABSTRACT

Raphanus sativus L. commonly known as Muli, belongs to the family Brassicaceae. Production of Reactive Oxygen Species (ROS) causes various diseases and cellular anomalies in human beings. Antioxidants inhibit generation of reactive species, or scavenge them, or raise the levels of endogenous antioxidant defenses. Keeping in view of the above beneficial effects of Muli, we shought to analyse the phytochemicals present in the quath, aqueous and methanolic extract. We collected Muli from the local market of Jhansi and washed with tap water and finally with distilled water. After drying the radish leaves at room temperature, it was crushed and was used for aqueous and methanolic and quath extractions. Phytochemical analysis of the leaves and roots of *R. sativus L.* var niger had most of the important phytoconstituents like Alkaloids, Tannins, Cardiac glycosides, Terpenoids, Flavonoids, Steroids are present in the methanolic extract of radish leaves while in quath and aqueous extract Alkaloids, Flavonoids, Glycosides, Tannin & Phenolic compounds, Saponins, Amino acids and Terpenoids are present in most of the test. As compared to leaves, radish root shows less phytochemical constituents in all the extract. We observe the antioxidant activities in both radish leaves and roots. We conclude that there was no effect of concentration on phytochemical constituents. Radish leaves have more phytochemical constituents while radish roots have less. Further, radish leaves show more antioxidant activities as compared to roots. However, methanolic extraction is superior to aqueous extraction for the study of phytochemical constituents.

Keywords: *Raphanus sativus,* Phytochemical components, Medicinal plants, Antioxidant, TLC

INTRODUCTION

Medicinal activities of plants have long been associated with the production of secondary metabolites which include alkaloids, flavonoids, tannins, terpenoids, coumarins, etc. Phytochemicals are bioactive chemicals, synthesized naturally in all parts of the plant body. They are called as secondary metabolites because the plants that produce them may have little need for them. Secondary metabolites are known to show curative activity against several ailments in man and other animal. These products help plant to carry out various activities like defense and pollination. However, their antioxidant, antimicrobial and other medicinal properties are widely exploited for the benefit of mankind regarding healthcare. Certain biological assays are conducted in order to assess the phytochemicals and antimicrobial potentials of a plant[1]. Herbal products are suitable for treating a wide range of infections and diseases.

Raphanus sativus L. plays a very important role in treating illness. *Raphanus sativus* that belongs to family Brassicaceae has been used as a medicinal plant from a long time It is known as Mooli or Muli in Hindi & Punjabi. It has laxative effects on intestine and acts as an appetizer[2], used for curing liver dysfunction and poor digestion[3], acts as antioxidant[4], anti-tumorigenic[5], anti-diabetic[6], and anti-proliferative[7]. It is also very well known for its use in the treatment of bronchitis and diarrhea[8]. Resistance to anti-infective drugs by bacteria is a growing problem. Thus, to reduce the use of antibiotics, better understanding of the mechanisms of bacterial resistance and develop new drugs whether synthetic or natural are urgent need[9,10]. The antimicrobial agents from plant sources may act through diverse mechanisms, and could therefore be of therapeutic importance in the treatment of bacterial infections[11]. It is also very well known for its use in the treatment of gynecological disorders and in jaundice. Its seeds have been found to be useful in urinary diseases in Bechar district of South West of Algeria. It is also found to have phyto remediation ability for contaminated soils.

Vitamin C is found in large quantity in radish which helps to build tissue, blood vessels, bones and teeth. Other vitamins (B, K, folate, etc.) and minerals (potassium, magnesium, calcium, iron, zinc, copper, sodium, and phosphorous) are also found. Vitamin C is known for its antioxidant activity. Further, Radish is known for fiber and roughage along with plenty of water. Since, it contains plenty of roughage therefore it is very effective in the treatment of piles and constipation. The juice of radish helps in indigestion and is the excellent detoxifier, removes bilirubin from the blood and very useful for jaundice patients. Radish not only removes bilirubin but also stops its production. It helps to purify blood and increases the oxygen carrying capacity of blood. Radish is a natural diuretic. It helps to prevent urinary tract infection and burning sensation of urinary problems.

Oxidative stress results in an increased production of reactive oxygen species (ROS) and lead to unlimited oxidation of proteins and membrane lipids or may cause DNA damage and other serious diseases like ageing, cancer, diabetes, atherosclerosis and other neurodegenerative diseases [12,13]. In certain circumstances protective mechanism carried out by defense system can be exhausted that leads to elevated levels of peroxidation products. Antioxidants inhibit generation of ROS, or scavenge them, or raise the levels of endogenous antioxidant defenses. Radish

contains isothiocyanate antioxidant compound called sulforaphane. Sulforaphane has proven role against prostate, breast, colon and ovarian cancers by virtue of its cancer-cell growth inhibition, and cytotoxic effects on cancer cells. Isothiocyanates fights against different types of cancer viz. oral cancer, colon cancer, intestinal cancer, kidney cancer and stomach cancer. A study was conducted by Jawaharlal Nehru Technological University showed that radishes genetically alter the path of cancer cells.

Preliminary qualitative test[14] is useful in detection of bioactive principles and subsequently may lead to drug discovery and development[15]. Further, phytochemical screenings of medicinal plants are very important in identifying new sources of therapeutically and industrially important compounds. It is also used in pharmaceutical and nutraceutical products of commercial importance. Encouragement for conservation and cultivation of herbal fauna can play an important role in livelihood enhancement of rural population[16]. The presence of bioactive compounds indicates the medicinal value of the plants. Antioxidants and antimicrobial/ antibacterial properties of various extracts from many plants have recently been of great interest in both research and the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants and antimicrobials with natural ones[17]. Therefore, in the present study, qualitative phytochemical analysis was carried out in the leaves and roots of radish collected from the local market of Jhansi city. Jhansi is in the Bundelkhand region of Uttar Pradesh. Thin layer chromatography was also performed Extraction was carried out by different methods. The medicinal properties such as anti-oxidant activity of radish was evaluated.

MATERIALS AND METHODS

Collection of Plant Materials

Raphanus sativus was collected in the month of January from local market of Jhansi (U.P). Firstly, the collected plant material was washed with tap water for 3-4 times and then with de-ionized water for two times. After washing, plants were kept in the dark for drying at room temperature and under the constant observation to avoid any contamination. After drying, the roots and leaves were crushed with the help of electric grinder. Powdered sample was stored for further use.

Extraction Procedure

Extraction was done by two methods i.e. Aqueous and Methanolic extraction.

Aqueous Extract

Different concentration of dry powder i.e. 5gm and 10 gm was taken in conical flasks having equal amount (100ml) of de-ionized water. Both the flasks were heated at 90°C in water bath for 1 hour. After 1 hour, flasks were taken out from water bath and kept at room temperature for cooling purpose. Then the extract was filtered with the help of filter paper and stored at 4°C.

Methanolic Extract

The powdered material was extracted with absolute 80% methanol using Soxhlet apparatus. Different concentration of plant material and solvent were taken. After filling the soxhlet apparatus with plant material and solvent it was run at 60-80°C until it gets colorless and continuously flow of water to cool down the condenser. Finally, the extract was collected in air tight bottles and stored at 4°C.

Phytochemical Analysis

Detailed phytochemical analysis was carried out for all the extracts as described elsewhere[18] with some of modifications**.**

Thin layer chromatography

Each of the extracts was to begin with, checked by thin layer chromatography (TLC) on analytical plates over silica gel-G of 0.2 mm thickness. These plates were developed in Butanol: Acidic acid: Water having a ratio of 2:1:1. The developed TLC plates were air dried followed by hot air oven for 20 minutes. Freshly prepared 0.2 % ninhydrin solution was used to detect the bands on the TLC plates.

The movement of the spots were expressed by its retention factor (Rf).

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Antioxidant activity

The total antioxidant capacity of the methanolic and aqueous extract of *Raphanus sativus* were evaluated by the phosphomolybdenum reduction assay method according to the procedure described by Prieto *et al*[19]. The assay is based on the reduction of Mo (VI) to Mo (V) by the methanol extract of different part of garlic and subsequent formation of green phosphate/Mo (V) complex at acid pH. One mL of various concentrations (3- µg/mL) of the extract was combined with 1 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. BHT was used as a standard. A typical blank solution contained 3 ml of the reaction mixture and the appropriate volume of the same solvent used for the samples/standard. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer.

RESULTS

Phytochemical investigation of *Raphanus sativus* L. shows the presence of many phytochemicals viz. Alkaloids, Reducing sugar, Flavonoids, Glycosides, Cardiac glycosides, Tannins, Saponin, Protein, Amino acid, Terpenoids and steroids etc. The radish leaves show most of the phytoconstituents are present in quath, aqueous and methanolic extracts. Alkaloids is present in all extracts i.e. aqueous, methanolic and quath. Carbohydrate is absent by Barfoed's test in all extracts while Molisch test shows positive results only in quath but absent in both aqueous and methanolic extract. Reducing sugar is present in all extracts by Benedict's test while it is absent by Fehling's test. Flavonoids are present in radish leaves while roots show negative results. Test for Glycosides shows variable results by different tests. Cardiac glycosides are present in aqueous and methanolic extract while absent in quath. Tannin and phenolic compounds are present in all extracts by ferric chloride test in radish leaves, but it is absent in roots. Saponin is present in all the extracts. Amino acid and protein is present by ninhydrin test in all extracts except quath while it is absent in biuret test. Terpenoids is present in all the extracts while steroids are present only in methanolic extract. Ninhydrin test for amino acids and protein shows mostly positive results. However, Biuret test shows negative results in all the extracts of roots & leaves. Tests for terpenoids and steroids shows

variable results (Table 1). Radish leaves as well as roots shows antioxidant activities.

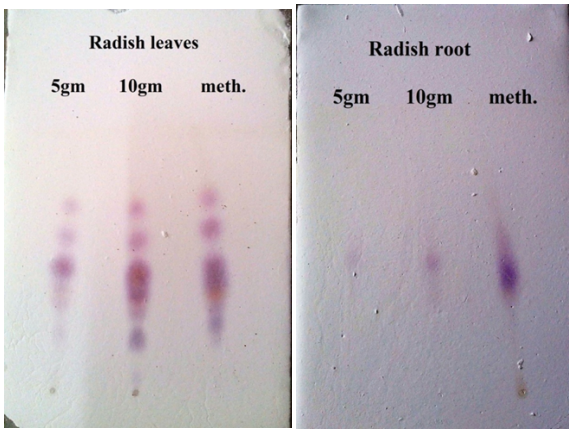
Thin layer chromatography

The thin layer chromatography of sample shows different spots. There are 6 spots are present in methanolic extract of radish leaves having Rf values 0.25, 0.37, 0.41, 0.47, 0.64, 0.74 respectively from spot 1 to spot 6. Aqueous extract of 10 gm of radish leaves shows 7 spots having Rf values 0.05, 0.23, 0.32, 0.41, 0.47, 0.58, 0.70 respectively from spot 1 to spot no 7. In aq. extract of 5gm of radish leaves, 6 spots are present having Rf 0.25, 0.39, 0.43, 0.47, 0.58, 0.70 respectively There are 2 spots are found in methanolic extract having Rf 0.38, 0.44 respectively. In aq. extract (10gm) of radish root 2 spot are found having Rf 0.42, 0.44 respectively. In aq. extract (5g) only one spot is found having Rf 0.5.

Table 1: Phytochemicals analysis of aqueous and methanolic extracts of the *Raphanus sativus* leaves & roots

Sl. No	PHYTOCHEMICAL TEST	RADISH LEAVES				RADISH ROOTS			
		QUATH	AQUEOUS		METHANOLIC	QUATH	AQUEOUS		METHANOLIC
			5 gm	10 gm			5 gm	10 gm	
1.	**TEST FOR ALKALOIDS**								
	(a)Mayer's test	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve
	(b)Wagner' test	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
	(c)Hager's test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
2.	**TEST FOR CARBOHYDRATE**								
	(a)Molisch test	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	(b)Barfoed's test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3.	**TEST FOR REDUCING SUGAR**								
	(a)Fehling's test	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
	(b)Benedict's test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
4.	**TEST FOR FLAVONOIDS**								
	(a)Alkaline reagent	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
	(b)Lead acetate	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
	(c)Ammonia test	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
5.	**TEST OF GLYCOSIDES**								
	(a)Borntrager test	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve
	(b)Legal's test	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	(c)10% NaOH test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6.	**TEST OF CARDIAC LYCOSIDES**								
	(a)Keller killani test	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
7.	**TANNIN AND PHENOLIC TEST**								
	(a)Ferric chloride test	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
	(b)Lead acetate test	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	(c)Dilute iodine test	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	(d)Ferric chloride 10%	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
	(e)Hydrolysable tannins	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
8.	**TEST FOR SAPONIN**								
	(a)Saponin test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
9.	**AMINO AND PROTEIN**	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	(a)Ninhydrin test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	(b)Biuret test								
10	**Test for terpenoids**	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve
	Test for steroids	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve

("+" = Positive; "-" = Negative; Aq. Ex.= Aqueous Extract; Met. Ex. = Methanolic Extract)



(A) **(B)**

Figure 1: TLC plate showing spots of different solvent extracts of leaf & root of *Raphanus sativus* (A: Leaves; B: Root)

DISCUSSION

Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design[20].

The phytochemical screening of methanolic extract of root revealed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, flavonoids, tannins and polyphenols[21]. Devaraj et al. reported that the ethanolic extract of its leaves and its fractions showed the presence of tannins, carbohydrates, proteins, alkaloids, saponins, flavonoids and glycosides in phytochemical screening[22]. It has been reported that the methanolic extract of its roots showed the elevated level of antioxidant enzymes in gentamycin induced nephrotoxicity in rats[23]. Meera *et al.* studied the phytochemical nature of aqueous and methanolic extracts of leaves of radish. The preliminary phytochemical screening of the extract showed the presence of tannins, alkaloids and flavonoids in the extracts[24]. Several other workers have also studied the antioxidant activity and phytoconstituents of various extracts of radish leaf and aerial parts. In a study by Reddy *et al.,* ethanolic extract of its leaves showed 45-50% DPPH radical inhibition at 200µg/ml concentration[25]. In a study by Beevi *et al.*, aerial parts of radish were found to possess potent antioxidant and radical scavenging activity as well as it was screened for polyphenolic content by HPLC. Methanolic and acetone extracts of *Raphanus sativus* leaves were found to have total polyphenolic content of 86.16 and 78.77 mg/g dry extract, respectively. It was also found to scavenge free radicals effectively with IC50 value of 31 and 42 mg/ml in case of DPPH radical. Hence it was regarded as a potential source of natural antioxidants[26].

The members of family Brassicaceae are rich in phytochemicals [27,28] and phytochemical analysis revealed the presence of tannins, saponins, flavonoids, phlobatannins, anthraquinones, carbohydrates, reducing sugars, steroids, phytosterol, alkaloids, amino acids, terpenoids, cardiac glycosides and chalcones in *R. sativus* niger leaves of methanolic extract[29]. In our study of pytochemical analysis of methanolic extract of radish leaves showed alkaloids, reducing sugar, flavonoids, glycosides, cardiac glycosides, tannins, phenolic compounds, saponin, amino acids, terpenoids and steroids. Almost all phytochemicals are present in our extract were identified either by one or other methods of tests. The aqueous extract of its root was found to show the presence of alkaloids, glycosides, triterpenoids and steroids, but carbohydrates, reducing sugars, flavonoids, tannin, phenolic compounds, saponin, proteins and amino acids were absent[30]. While in our study alkaloids, reducing sugar, tannins, saponin and amino acids are present.

Valko *et al.* reported that one of the most actively studied properties of phenolic compounds in general and flavonoids are their ability of conferring protection against oxidative stress[31]. Thus in this study, the presence of flavonoids and phenolic compounds in the leaves extract could be considered responsible for conferring greater antioxidant ability to the leaf extract as compared to its roots extract in which both these compounds were absent. Flavonoids are a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and have strong anticancer activity[32,33]. It also helps in managing diabetes induced oxidative stress. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Further, terpenoids are also known to possess antimicrobial, antifungal, anti-parasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties. [34-35] In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well [36].

CONCLUSION

Phytochemical investigation of *Raphanus sativus* L. shows the presence of Alkaloids, Reducing sugar, Flavonoids, Glycosides, Cardiac glycosides, Tannins, Saponin, Protein, Amino acid, Terpenoids and steroids etc. Methanolic extract was more effective than water extract because phytochemicals shows positive result in methanolic extract in more amount. There was no effect of concentration on the phytochemical constituents. Mostly, results are same in aqueous and quath extracts so we conclude that no need to dry the green leaves. Result depends upon the solvent as well as the method of test we apply. The plant study reveals that there were more phytochemicals are present in radish leaves rather than radish roots. Carbohydrate is present more amount in radish roots and less amount in radish leaves. Flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. Glycosides play an important role in lowering the blood pressure. They are also used in treatment of congestive heart failure and cardiac arrhythmia. The extract contains phytochemicals like phenols and flavonoids that cause inhibition, this property of plant may be important in preventing oxidative stress related diseases.

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