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Total phenolic content, antioxidant activity, alpha-amylase inhibitory activity and antibacterial activity of radish seed and rapeseed

Bibek Khatiwada*, Sobita Kunwar, Ashmita Dhakal, Ansu Joshi, Abdul Rehman Miya, Pradeep Subedi
Department of Biochemistry, Universal Engineering and Science College, Chakupat, Lalitpur, Nepal

Abstract
Natural products are considered as potential sources of pharmaceutical agents and/or as sources of lead compounds in drug development. In this study, two species of plant seeds were analyzed which are: Radish (*Raphanus sativus* L.) Rapeseed (*Brassica napus* L.) The present study was conducted to evaluate the the quantity of phytochemicals present, antioxidant activity, α-amylase inhibitory activity, and antibacterial activity of those two edible seeds. The plants extracts were obtained using methanol as a solvent. The total amount of phenolic content of different plant extracts was determined by Folin Ciocalteu method. The total flavonoids in the plant extract were estimated using the aluminum chloride colorimetry method. Antioxidant activity was analyzed by DPPH assay. Alpha-amylase inhibitory activity was determined by modified method described by the Worthington Enzyme Manual. The antibacterial activity was carried out by disc diffusion assay. The results revealed that the methanolic extract of rapeseed (1000µl) extract exhibited the highest radical scavenging activity with IC50 value of 323.30 mg/ml. Radish seed showed the highest alpha amylase inhibition activity with IC50 value of 1332.99 µg/ml. Both seeds showed significant antibacterial activity against six pathogenic bacterial species. Results obtained in this study indicated the promising medical potential of these two plant species.

Keywords: antioxidant, phenols, alpha-amylase, DPPH, antibacterial

1. Introduction
Phytochemicals are non-nutritive components present in a plant-based diet. Phytochemicals are found to possess protective or disease-preventing effects [1]. They have been associated with protection from and/or treatment of chronic diseases such as heart disease, cancer, hypertension, diabetes and other medical conditions [2, 3].

Antioxidants are those substances which have ability to scavenge the free radicals due to their redox hydrogen donators and singlet oxygen quencher. Phytochemicals such as phenols, flavonoids and tannins are found to exert antioxidant potential [4]. Though, synthetic antioxidants are available in the market, the preference is still given to natural antioxidants because they are considered safer and they have lower side effects [5].

A-Amylase is one of the key enzymes involved in the breakdown of starch into absorbable glucose molecules [6]. Inhibiting these enzymes help in reducing post-prandial glycemia by reducing the speed of glucose absorption and therefore can be an important strategy in the management of hyperglycemia linked to type II diabetes [7].

In this study, two plants seeds – radish seeds and rapeseeds were taken. Radish and rapeseed both belong to the family Brassicaceae. Various studies have found that plants belonging to this family contain a unique group of compound called glucosinolates. When enzymatically hydrolysed, glucosinolates yield isothiocyanates and give a pungent taste [8]. Organic isothiocyanates are a very important group of bio-active compounds which are found to exert significant anticancer properties [9]. In the current study, radish seeds and rapeseeds were analyzed for their antioxidant, alpha-amylase inhibitory and antibacterial potential.

2. Materials and methods
2.1 Chemicals and Standards
The chemicals used were methanol (Thermo-Fisher Scientific, India), DPPH and ascorbic acid (Sigma Aldrich, USA). All other chemicals used were of the highest commercially available grade. For absorption measurement, double beam U-2800 UV-visible spectrometer, HITACHI, Japan, was used.

2.2 Plant material and preparation of extract
Radish seeds (*Raphanus sativus* L.) cv. Chalis Dine and Rapeseeds (*Brassica napus* L.) cv. Preeti were purchased from Central Vegetable Seed Production Center in Khumaltar Lalitpur, Nepal. These different dry seeds were grinded in a blender to fine particles, which increased the surface area for extraction thereby increasing the rate of extraction. Twenty five gram of the fine powder of the radish seeds and rapeseeds were placed 250 ml (sample to solvent ratio of 1:10 (w/v) solvent to dry weight ratio has been used) of methanol as solvent. They were placed in two different conical flasks and stored at room temperature for 72 hours. The extracts were filtered through Whatman No. 1 filter paper and were evaporated to dryness using rotary evaporator at a much reduced pressure. This was followed by the dilution of the dry crude extract (concentrated using a rotary evaporator) with mother solvent to obtain a stock solution of 100mg/ml from

which a series of dilutions were made as required.

2.3 Determination of Total Phenolics

The total amount of phenolic content of different plant extracts was determined by Folin-Ciocalteu method [10]. To 1 ml Folin-Ciocalteu's reagent, previously diluted (1:4), was added to 1 ml of test solution of concentration 1 mg/ml. To the mixture, 4 ml of sodium carbonate (75g/L) and 10 ml of distilled water were added and mixed well. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000 g for 5 min and the absorbance of the supernatant was taken at 760 nm. A standard curve was obtained using various concentrations of Gallic acid. All the determinations were done in triplicate and the total phenolic content were expressed as Gallic acid equivalents (GAE).

2.4 Determination of Total Flavonoids

The total flavonoids in the plant extract were estimated using aluminum chloride colorimetry method [10]. In this method 1 ml of sample from concentration 1 mg/ml was added to 2 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solution. The absorbance was then taken at 440 nm after 2.5 h incubation at room temperature. All the measurements were done in triplicate and the total flavonoid content were expressed as Rutin equivalent (RE).

2.5 DPPH Radical Scavenging Activity

1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) radical scavenging activity was measured according to the method of Pokharel *et al*. 2015 with slight modifications [10]. 600 µM DPPH was made by dissolving DPPH powder in methanol and used as stock solution. Plant extracts were taken in various concentrations: 1000,500, 250, 125, and 62.5 µg/ml. An aliquot of 0.5 ml of 600 µM DPPH solution and 1 ml of plant extract at various concentrations were mixed and incubated at 25°C for 30 minutes in dark and absorbance of the test mixture was read at 517 nm using a spectrophotometer against methanol only blank. Absorbance of DPPH control containing only 1 ml of methanol in place of the extract was also measured. All experiments were performed thrice and the results were averaged. Ascorbic acid was used as a standard. Percentage inhibition was calculated using the following expression;

$$\% \text{ Inhibition} = (A_{control} - A_{sample})/A_{control} \times 100$$

Where, $A_{control}$ and A_{sample} stand for absorbance of the control and absorbance of tested extract solution, respectively.

2.6 Assay for α-Amylase Inhibition:

Alpha-Amylase inhibitory activity was determined by the method described by the Worthington Enzyme Manual with slight modification [11]. A total of 500 µl of extract and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 500 µl α-amylase solution (1.0 U/ml) was incubated at 25 °C for 10 min. After pre-incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped by adding 1.0 ml of DNS (Dinitrosalicylic acid) color reagent. The test tubes were then incubated in a boiling water bath for 5 min and

cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance was measured at 540 nm. The α-amylase inhibitory activity was calculated according to the equation given below:

$$\text{Inhibition (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100$$

Where $A_{control}$ was the absorbance of the control (without extract); A_{sample} was the absorbance in the presence of extract.

2.7 Antibacterial assay

Antibacterial assay was done by the disc diffusion method [12]. The test pathogens (100 µL/plate) were spread on Muller Hinton agar plates. The filter paper discs (6 mm in diameter) were impregnated with the crude extract at the concentration of 100 mg/disc and then placed onto the agar plates previously inoculated with the tested microorganisms. The tested microorganisms included *Proteus vulgaris*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The plates were incubated at 37 °C for 24 h to allow the growth of the microorganisms. The diameters of the zones of inhibition were measured in millimeters using a calibrated scale. All the tests were repeated triplicate. Gentamicin was used as standard at the dose of 1 mg/disc and methanol was used as negative control.

3. Results

3.1 Total Phenolic Content

The content of phenolic compounds in different seed extracts was determined from the regression equation of the calibration curve (y= 0.0015x- 0.03, R^2= 0.9987). A calibration curve was drawn by using different concentration of gallic acid (not shown here). The amount of phenolic compounds was expressed as gallic acid equivalent (GAE) in mg/g dry weight of seed extract and is shown in Table 1 below. Total phenolic content was higher in rapeseed than radish seed.

3.2 Total Flavonoid Conten

The flavonoid content in different seed extracts was determined from the regression equation of the calibration curve (y= 0.0018x- 0.063, R^2= 0.9994). A calibration curve was drawn by using different concentration of rutin (not shown here). The amount of flavonoids was expressed as rutin equivalent (RE) in mg/g dry weight of plant extract and is as shown in Table 1. Total flavonoid content was also higher in rapeseed than radish seed.

Table 1: Total amount of phenols, flavonoids in radish seeds and rapeseeds

Sample	Total phenolic content mg GAE/g plant extract	Total flavonoids mg RE/g plant extract
Radishseed	86.09 ± 2.63	165.19 ± 3.08
Rapeseed	98.31 ± 4.29	202.57 ± 3.71

(All the data are represented in mean±standard deviation, n=3)

3.3 DPPH Radical Scavenging Activity

DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of plant extracts. In vitro antioxidant studies of the different extracts, the extent of DPPH radical scavenging at different concentrations (62.5, 125, 250, 500, 1000 µg/ml) of radish seed and rapeseed extracts was measured, with ascorbic acid as the standard. The radical scavenging effect was found to increase generally with increasing concentrations Table 2.

The antioxidant activity of various extracts were expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. The lower the IC50 value, the higher the radical scavenging activity. The radish seed was found to have higher antioxidant activity than rapeseed as shown in Fig. 1.

Table 2: Antioxidant activity by DPPH assay

	Inhibition (%)				
Samples	**62.5(µg/)**	**125(µg/m)**	**250(µg/ml)**	**500(µg/ml)**	**1000(µg/ml)**
Radish Seed	30.26±1.36	43.07±0.92	61.31±2.21	74.27±2.85	89.12±0.86
Rapeseed	25.04±2.42	42.36±3.26	59.15±1.28	68.43±2.38	75.21±1.41
Ascorbic Acid	95.28±1.36	95.86±2.21	96.31±0.97	96.54±1.67	97.26±1.06

(All the data are represented in mean± standard deviation, n=3)

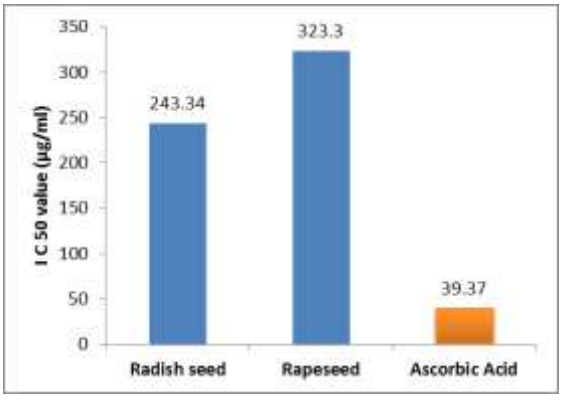

Fig 1: Antioxidant activity of radish seed and rapeseed by DPPH assay

3.4 Assay for alpha- Amylase inhibition

Alpha-Amylase inhibitory activity was measured for radish seed and rapeseed extracts in different concentrations (500, 1000, 1500, 2000, 2500 µg/ml) with Acarbose (a known alpha-amylase inhibitor) as a standard. The alpha-amylase inhibitory activity was found to increase generally with increasing concentrations (Table 3).

The alpha-amylase inhibitory activity of various extracts were expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extracts that inhibits the activity of alpha-amylase enzyme by 50%. The lower the IC50 value, the higher the alpha- amylase inhibition activity. Alpha- amylase inhibitory activity was found to be higher in rapeseed than radish seed.

Table 3: Alpha-amylase inhibition

	Inhibition (%)				
Samples	**500(µg/ml)**	**1000(µg/ml)**	**1500(µg/ml)**	**2000(µg/ml)**	**2500(µg/ml)**
Radish Seed	16.92±2.43	45.14±3.78	59.06±3.39	74.18±2.67	82.76±1.34
Rapeseed	30.67±2.73	53.18±4.27	64.21±2.13	86.43±5.08	91.23±5.33
Acarbose	81.17±2.31	95.67±2.06	97.04±1.67	99.21±0.91	99.58±0.67

(All the data are represented in mean± standard deviation, n=3)

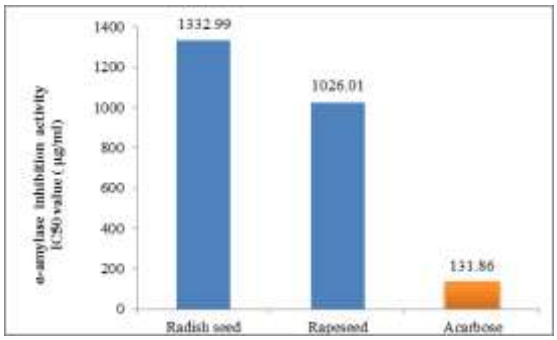

Fig 2: α-amylase inhibition activity of radish seed and rapeseed

3.5 Antibacterial Assay

The result of antibacterial activity of radish seed and rapeseed is shown in Table 4. Radish seed was the most effective against *Pseudomonas aeruginosa* showing maximum zone of inhibition of 14±2 mm when 100 mg extract was used. Rapeseed was the most effective against *Escherichia coli* showing maximum zone of inhibition of 13±1.15 when 100 mg extract was used. Both the extracts were least effective against *Shigella dysenteriae* than other strains used.

Table 4: Antibacterial activity of radish seed and rapeseed extract

Samples	**Amount of Sample**	**Zone of inhibition (mm)**					
		Proteus vulgaris (Gram –ve)	**Salmonella typhi (Gram –ve)**	**Shigella dysenteriae (Gram–ve)**	**Pseudomonas aeruginosa (Gram -ve)**	**Staphylococcus aureus (Gram +ve)**	**Escherichia coli (Gram –ve)**
Radish Seed	100 mg	7 ± 1.15	9 ± 2.51	6 ± 1.53	14 ± 2	10 ± 1	11 ± 1.53
Rapeseed	100 mg	10 ± 2	7 ± 2	6 ± 1	9 ± 1.53	8 ± 1	13 ± 1.15
Gentamicin	2 mg	22 ± 1	20 ± 1.53	22 ± 2	24 ±1	19 ± 2.51	21 ± 1.53

(All the data are represented in mean±standard deviation, n=3)

4. Discussions

Total phenolic and flavonoid contents of the methanolic extracts of the radish seed and rapeseed were evaluated. Phenolic and flavonoid compounds are commonly reported in plants and they are known to exert various biological activities, including antioxidant activity [13, 14] as well as possess antibacterial properties [15, 16]. Phenolic compounds are also known to possess alpha-amylase inhibitory potential [17]. Thus, the antioxidant, alpha-amylase inhibition and antibacterial activities of seed extracts of radish and rapeseed may be attributed to their high phenolic and flavonoid contents. Earlier studies have confirmed that some of the identified compounds in radish and rapeseed extract indeed possess antioxidant activities and anticancer activities [18, 19, 20]. Several studies have reported a strong and significant correlation between the scavenging activity and total phenolic compound, as well as the flavonoid content and its significant contribution toward the total antioxidant activity [21]. DPPH radical scavenging activity assay assesses the capacity of the extract to donate hydrogen or to scavenge free radicals. Data from our study show that, radish and rapeseed were found to have high antioxidant potential which might be due to high phenolic and flavonoid contents.

The present study evaluated the ability of radish seed and rapeseed extracts to inhibit the activity of α-amylase, a digestive enzyme secreted from the pancreas and salivary gland. α-Amylase is involved in important biological processes such as digestion of carbohydrates. Many crude drugs as well as naturally occurring polyphenols are known to inhibit α-amylase activity [22]. Natural α-amylase inhibitors are beneficial in reducing post-prandial hyperglycemia by delaying the digestion of carbohydrates and, consequently, the absorption of glucose. Methanolic extract of both seeds in our study were found to possess significantly high alpha-amylase inhibitory potential.

The discovery of novel antimicrobial metabolites from medicinal plants is an important alternative to overcome the increasing levels of drug resistance by human pathogens. Due to the world's urgent need for new antibiotics and chemotherapeutic agents, growing interest is taken into the research on the chemistry of medicinal plants. In our study, both radish seed and rapeseed extracts showed significant antibacterial activity against six microorganisms (Table 4).

5. Conclusion

Hence, from the present study it can be concluded that radish seed and rapeseed can be considered as important pharmaceutical agent having high content of phenolics and flavonoids and high antioxidant potential. The antioxidant activity of the studied plant was higher which could be due to the higher content of phenols and flavonoids. The α-amylase inhibition activity as well as antibacterial activity of radish seed and rapeseed were also significantly high.

6. Conflict of Interest Statement

The authors declare that they have no competing interests.

7. Acknowledgment

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