**ABSTRACT**

Medicinal plants and vegetables are promising source of antioxidant products. The purpose of this study is to evaluate the phytochemicals and antioxidant activity of leaves and roots of *Raphanus sativus* of Saudi origin. Various phytochemicals were screened in n-hexane, chloroform, ethyl acetate and aqueous fractions of leaves and roots of *Raphanus sativus* using standard protocols and IR screening method. Total phenolic (TPC) and flavonoid (TFC) contents were assessed by Folin-Ciocalteau and aluminium chloride methods respectively. The antioxidant activity was evaluated by DPPH antioxidant protocol, using trolox as standard. Results demonstrated that *Raphanus sativus* chemically characterized by the availability of various constituents such as flavonoids, steroids, saponins, tannins and carbohydrates at different levels in fractions of leaves and roots of *Raphanus sativus* and the absence of cardiac glycosides, anthraquinones and alkaloids which was further confirmed using FTIR analysis. TPC was ranged from (8.92±1.01) and (211.80±1.57) mg GAE/g extract and TFC was ranged from (0.03±0.03) and (11.57±0.60) mg QE/g extract for leaves and roots respectively. Due to the high phenolic and flavonoid content in aqueous and ethyl acetate fractions of leaves and roots extracts, results demonstrated high antioxidant activity with IC50 of (56.3±1.3) and (69.7±1.8) for aqueous fractions and (47.2±1.5) and (58.7±0.7) for ethyl acetate fractions of leaves and roots extracts respectively. Study revealed that ethyl acetate and aqueous fractions of leaves and roots of *Raphanus sativus* could develop a potential natural antioxidant herbal remedy. The study recommends future investigation to isolate and identify the bioactive secondary metabolites in *Raphanus sativus*.

**Key words:** *Raphanus Sativus*, Folin-Ciocalteau, DPPH, Total phenolic, Total flavonoid.

**INTRODUCTION**

Reactive oxygen species (ROS) are highly reactive substances which may bring about deep harms to tissues and consequently lead to serious degenerative conditions, such as ageing disorders, cancer, cardiovascular damage and diabetes. Hence, living organisms are gifted with defense mechanisms but unquestionably it is needed for additional support of external antioxidants to express more arsenals to fight. Antioxidants constituents are reputed to inhibit the harmful effects of ROS. However, synthetic antioxidants are available to help but they may have toxic and adverse effects. Hence, the development and exploitation of natural antioxidants are anticipated. Recently, medicinal plants including vegetables are promising source of antioxidant agents because of their potency and diversity in chemical structure as compared to synthetic compounds. The area of Arabian Peninsula (AP) comprises hundreds of species of medicinal plants and vegetables, The Kingdom of Saudi Arabia as part of AP, is rich biodiversity flora. Most of those native and cultivated plants are reputed for their higher contents of various bio-constituents and wide range of therapeutic benefits against different infectious diseases. *Raphanus sativus* L. (Brassicaceae) commonly known as radish, is an annual herb with white or brightly pink colored edible roots. It is commonly and traditionally used as a vegetable or appetizer in salads. It grows and cultivated worldwide including Saudi Arabia. Leaves and peel of root are always discarded which may possess potent biological properties. A literature survey indicated that, many studies revealed the identification of phytochemicals and biological activities including; anticancer, antimicrobial, antidiabetic and diuretic activities of roots and leaves of *Raphanus sativus* of many origins especially Indian origin. Different habitats and environmental conditions could alter the bio-synthesis and machinery pathways in plant species. Hence, and based on the concept of different origins of the same plant may lead to different constituents or at least different concentrations of compounds. The present work is to investigate and highlight the phytochemical profiling including determination of total phenolic and flavonoid contents and radical scavenger activity of various fractions of different polarities of leaves and roots of white radish grown in Eastern Province of Saudi Arabia to categorize the most active fractions for future investigation to isolate and identify the bioactive secondary metabolites.

**MATERIALS AND METHODS**

**Materials**

Radish material (*Raphanus sativus*) was collected from local farm, Al-hasa, Eastern Province, Saudi Arabia. Insights into Screening of Secondary Metabolites, Phenolic and Flavonoid Contents and Antioxidant Activity of *Raphanus sativus* L. Cultivated in Eastern Province of Saudi Arabia. Pharmacogn J. 2022; 14(4): 313-318.
Pharmacognosy Journal, gives a brick-red color that indicates the presence of glycosides. After being heated on a water bath for about two minutes, it was mixed with Fehling's solution (A) and (B) and a mixture of the extract was shaken vigorously. The appearance of 1 cm froth for 15 minutes indicated the presence of saponins. To give to give 4.3 g and 2.4 g for leaves and roots respectively. The remaining aqueous fraction was subjected to further fractionation with chloroform (4×500 mL). The chloroform fractions were combined and concentrated to yield 9.5 g and 1.3 g, for leaves and roots respectively. The remaining aqueous fraction was lyophilized to give 2.4 g and 1.8 g for leaves and roots respectively.

Screening of secondary metabolites

Flavonoid

Extract of 2 ml mixture was mixed with of 1 ml 2 % NaOH. Deep yellow color was developed, which turned colorless on addition of 2 drops of concentrated sulphuric acid. This result indicates the presence of flavonoids.

Alkaloid

Extract of 0.5 ml was dissolved in 1.5 ml dilute Hydrochloric acid and filtered. The obtained filtrate was mixed with 2-3 drops of Dragendorff’s reagent and observed for five minutes. Dragendorff’s reagent produces an orange or orange-red precipitate indicates the presence of alkaloids.

Saponins

Extract of 1 ml was dissolved in 20 ml distilled water and mixture was shaken vigorously. The appearance of 1 cm froth for 15 minutes indicates the presence of saponins.

Steroid

Extract of 0.5 ml was dissolved in 5 ml chloroform and few drops of acetic anhydride and concentrated sulphuric acid were added from the side of the test tube. The upper yellow layer with green/blue color indicates the presence of steroids.

Tannins/Phenolics

About 2-3 drops of 5% ferric chloride solution was added to 0.5 ml of extract gives intense blue-greenish indicating the presence of tannins/phenolics.

Glycosides

Extract of 2 ml was mixed with Fehling’s solution (A) and (B) and mixture was heated on a water bath for about two minutes. After heating, it gives a brick-red color that indicates the presence of glycosides.
present in media of reaction. Consequently, the color was changed from violet to yellow indicating the presence of antioxidant. Different fractions were dissolved in 100 µl of methanol and placed in 96-well micro plate. The absorbance of was measured at 515 nm. Zero time was considered as Ab blank. Then, 100 µl of DPPH solution (concentration of 200 µM) was inserted into every well. The plates were kept 25°C for 30 min. Followed by, measurement of the absorbance again as Ab sample. The percentage of inhibition was measured using this equation:

\[
\text{% of inhibition} = \left(1 - \frac{\text{Ab sample} - \text{Ab blank}}{\text{Ab control} - \text{Ab blank}}\right) \times 100
\]

Where: Ab control is the absorbance of mixture (with DMSO and all other reactant without tested extracts). IC\text{\textsubscript{50}} was recorded as the sample concentration that is essential to produce inhibition of DPPH radical to be formed by 50%.

RESULTS AND DISCUSSIONS

Phytochemical screening of different fractions

The preliminary phytochemical screening of various fractions demonstrated the presence of different phyto-constituents such as flavonoids, saponins, steroids, tannins/phenols, glycosides and carbohydrates at different levels in different factions and the absence of cardiac glycosides, anthraquinones and alkaloids as shown in Table 1.

Fourier transform infrared spectroscopy (FTIR) screening

The spectra of various fractions showed bands approximately at 3400–3420 cm\(^{-1}\), related to stretching vibration of hydroxyl groups (OH of phenolic compounds) Other bands were exhibited at 2900-3000 cm\(^{-1}\) which could be assigned to stretching vibration of C-H bonds of aromatic skeletons like flavonoids or aromatic acids bands at 1650–1710 cm\(^{-1}\), probably related to stretching vibration of carboxyl groups, stretching vibration of C=C and C=O groups of aromatic ring deformations (flavonoids and phenolic acids. Bands at 1350–1550 cm\(^{-1}\) could be related to bending vibration of CH3, CH2, stretching vibration of flavonoids and aromatic rings. The bands at 1100–1260 cm\(^{-1}\) would be due to vibration of C-O group of polyhydroxy constituents, such as hydroxylflavonoids. Bands at 1010–1090 cm\(^{-1}\) would be related to alcohols and/or to C-O stretching ester groups. Bands at ~878 cm\(^{-1}\), probably related to aromatic ring vibration (Figure 1). These results also confirmed the results of phytochemical screening.

TPC assessment

TPC in various factions was calculated using the regression equation: 
\[y = 0.0357x + 0.0051, R^2 = 0.9702\]. The results were presented as the equivalence of milligrams of quercetin per gram of dried plant extract (mg QE/g) (Figure 3A). Results depicted that the amount of total flavonoid constituents differed from organ to organ and varies from (0.036±0.03) to (11.57±0.60) mg QE/g of dry extract. Similarly, to TPC, ethyl acetate fractions were the richest factions in TFC then aqueous fractions. Chloroform factions demonstrated the least amount of TFC compared to other factions. On the other hand, n-hexane factions hardly contained any flavonoid constituents (Figure 3B). The results demonstrated that leaves factions are richer in contents compared to roots factions.

Antioxidant activity (DPPH)

Besides, various factions were inspected for their radical scavenger potential using the DPPH free radical scavenging assay (Figure 4). Ethyl acetate faction showed marked scavenging activity (IC\text{\textsubscript{50}}: 47.2±1.5 and 58.7±0.7 µg/ml, for leaves and roots factions respectively), aqueous faction showed moderate activity (IC\text{\textsubscript{50}}: 56.3±1.3 and 69.7±1.8 µg/ml for leaves and roots factions respectively) and n-hexane and chloroform fractions demonstrated much weaker potentials with IC\text{\textsubscript{50}} above 100 µg/ml, all results were comparable to the standard trolox (IC\text{\textsubscript{50}}=22.7 µM).

CONCLUSION

The significance of herbal extracts as antioxidant agents gained merits being of low risks and higher efficiency compared with synthetic agents. The present work demonstrated that Raphanus sativus of Saudi origin have several secondary metabolites which have significant antioxidant activity specially the ethyl acetate and aqueous factions. Results could be credited to the higher TPC and TFC in these factions. This was confirmed from the elevated GAE/g and QE/g values. These distinguished results serve as evidence to recommend future investigation to isolate and identify the bioactive secondary metabolites in Raphanus sativus. Hence, to open the gate for further developing of some of Saudi origin food into natural antioxidant herbal remedy in the pharmaceutical industry.

Table 1: Preliminary phytochemical screening of various fractions of Raphanus sativus.

<table>
<thead>
<tr>
<th>Phyto-constituent/Fraction</th>
<th>LnH</th>
<th>LCH</th>
<th>LEA</th>
<th>LAQ</th>
<th>RnH</th>
<th>RCH</th>
<th>REA</th>
<th>RAQ</th>
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<tbody>
<tr>
<td>Flavonoids</td>
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<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Saponins</td>
<td>++</td>
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<td>+</td>
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<td>Steroids</td>
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<td>+</td>
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<td>Tannins/Phenols</td>
<td>-</td>
<td>-</td>
<td>++</td>
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<tr>
<td>Anthraquinones.</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Cardiac glycosides</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Carbohydrates</td>
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<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
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</table>

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**Figure 1:** FTIR spectra of; ethyl acetate fraction of leaves (A), ethyl acetate fraction of roots (B), aqueous fraction of leaves (C) and aqueous fraction of roots (D).

**Figure 2:** Calibration curve for gallic acid (A) and total phenolic contents (B) of various fractions of leaves and roots of *Raphanus sativus*. nH: n-hexane fraction, CH: chloroform fraction, EA: ethyl acetate fraction, AQ: remaining aqueous fraction, GAE/g: Gallic acid equivalence per gram of dried plant extract.

**Figure 3:** Calibration curve for quercetin (A) and total flavonoid contents (B) of various fractions of leaves and roots of *Raphanus sativus*. nH: n-hexane fraction, CH: chloroform fraction, EA: ethyl acetate fraction, AQ: remaining aqueous fraction, QE/g: quercetin equivalence per gram of dried plant extract.
Figure 4: Antioxidant activity (DPPH) of various fractions of leaves and roots of *Raphanus sativus*. nH; n-hexane fraction, CH; chloroform fraction, EA; ethyl acetate fraction, AQ; remaining aqueous fraction.

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GRAPHICAL ABSTRACT

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