Comparative study of antioxidant and anticancer activities and HPTLC quantification of rutin in white radish (*Raphanus sativus* L.) leaves and root extracts grown in Saudi Arabia

Abstract: The nutrient contents of *Raphanus sativus* L. (white radish) leaves (RSLs) and roots are known to have promising vital effects. We comparatively investigated the leaves and roots of *R. sativus* grown in Saudi Arabia to estimate the total phenol and flavonoid contents using the standard colorimetric methods. The antioxidant activity of RSLs and *R. sativus* roots (RSRs) was measured by 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. Both extracts’ cytotoxic activity was assessed by MTT assay against several human cancer cell lines (A549, HepG2, MDA-MB-231, and MCF-7). High-performance thin-layer chromatography was used to identify and quantify the biomarker compound rutin in both extracts. The total phenolic content in RSLs (125.3 mg of gallic acid equivalent [GAE]/g) was higher than that in roots (95.8 of GAE/g), whereas the flavonoid content (44.5 mg of quercetin equivalent [QE]/g) was almost double compared in RSR extract (24.4 of QE/g).

Similarly, RSLs exhibited a higher antioxidative activity than RSRs in both DPPH (IC$_{50}$ 216.8 vs 359.7 µg/mL) and ABTS (IC$_{50}$ 326.7 vs 549 µg/mL) models. RSLs also demonstrated the highest antiproliferative efficiency against all cell lines, with IC$_{50}$ values of 217–453 µg/mL. The reversed-phase-high-performance thin-layer chromatography results showed the presence of rutin (5.2 µg/mg) only in RSLs. Our study indicates RSLs as a promising source of bioactive compounds compared with roots.

Keywords: *Raphanus sativus*, rutin, HPTL, antioxidant, anticancer

1 Introduction

*Raphanus sativus* L., also popularly called as radish, is a commonly cultivated crop that belongs to the Cruciferous family and widely distributed and consumed worldwide [1]. The radish roots are the most well-known part of consumption, although other parts such as the stem and leaves are also used as vegetables by several populations [2]. Radish’s nutritional benefit comes from its valuable constituents, including high-fiber and low-fat contents and several essential vitamins and minerals [3]. In folk medicine, the different parts of radish are used for various purposes; for example, the roots are used to treat bladder pain and syphilis diseases, and the leaf juice is used as a laxative and diuretic [4]. Studies have reported several important activities, and properties of *R. sativus* leaves and roots (RSLs and RSR), including antimicrobial [1], antioxidant [5], antmutagenic [6], and anticarcinogenic effects [7]. Radish’s biological activities are due to the presence of several classes of phytoconstituents such as alkaloids, phenols, and flavonoid compounds [1,3,8–12].
Flavonoid compounds known for their nutraceutical and pharmacological properties are among the most important of these phytochemicals in *R. sativus*. Rutin, a flavonoid compound, represents flavonoids that naturally and widely occur in several fruits as well as vegetables. It possesses powerful antioxidant properties and several nutritional and beneficial effects that reduce the risk of several complex diseases [13,14]. Hence, it is imperative to investigate novel natural sources of rutin. Owing to its health-protective phytoconstituents contents, *R. sativus* has been receiving research attention from the scientific community. Therefore, we conducted a comparative study of the RSLs and RSRs grown in Saudi Arabia. The phenolic and flavonoid contents and the antioxidant and anticancer activities of both leaves and roots were comparatively investigated. To estimate and establish the presence of rutin in the leaves or roots of radish, we also conducted RP-high-performance thin-layer chromatography (HPTLC) to quantify rutin (Figure 1) in both plant parts.

![Figure 1: Total contents of phenols and flavonoids in RSLs and RSRs.](image)

2 Materials and methods

2.1 Collection of plant material

Field-grown radishes were bought from one of the farms which is located in the area of Al-Hayer, Riyadh, Saudi Arabia. They were stored in darkness at 5 ± 1°C before processing. The roots were separated from leaves, and both parts were washed in tap water to eliminate any surface contamination. All determinations were made using fresh products immediately after washing.

2.2 Preparation of extracts

Fresh RSLs and RSRs (200 g each) were extracted with 70% ethanol following the maceration procedure. Rotary evaporator was used to concentrate the liquid extract and 11 g of RSL and 9 g of RSR extracts were obtained, which were then stored in the refrigerator prior to its use.

2.3 Phytochemical analysis

2.3.1 Estimation of total phenol content

The RSL and RSR extracts’ total phenolic content (TPC) were estimated using a standard Folin–Ciocalteu method [15]. Briefly, 100 µL of 0.5 N Folin–Ciocalteu reagent was added to 500 µL of each extract. This was followed by a thorough mixing of all contents within the flask, after which 2.5 mL sodium carbonate was included. The mixture was incubated for 0.5 h. Finally, the phenolic contents’ absorbance was measured at 760 nm and the concentration was expressed as milligram gallic acid equivalent (GAE)/g of the extract.

2.3.2 Estimation of total flavonoids

The standard aluminum chloride spectrophotometric method previously described [16] was used to estimate the total flavonoid content (TFC). Briefly, 1 mL of 10% AlCl₃ was sequentially added to 1 mL of each RSL and RSR extract of 1 mg/mL concentration. The solution was then thoroughly mixed after which it was incubated for 30 min. After the incubation, the absorbance measurement was conducted in which the known quercetin concentrations were used to generate a standard calibration plot at 415 nm. The concentrations of flavonoids in RSLs and RSRs were estimated using the calibration plot, and it was expressed as milligram quercetin equivalent (QE)/g of the extract.

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

Estimation of the antioxidative activity of the RSLs and RSRs of *R. sativus* was conducted following the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as previously reported [17]. Various concentrations of RSL and RSR extracts (10, 50, 100, 500, and 1,000 µg/mL) were prepared. Next 0.5 mL of RSL and RSR extracts was mixed with DPPH (0.1 mM, 0.125 mL) and methanol (0.375 mL). This was followed by incubation for 0.5 h and then the
optical density was measured at $\lambda_{\text{max}} = 517$ nm, and ascorbic acid served as a positive control. The radical scavenging activity was computed using the given formula:

\[
\% \text{ of radical scavenging activity} = \frac{(\text{Abs control} - \text{Abs extract}/\text{Abs control}) \times 100.}
\]

The IC$_{50}$ values were generated from dose–response curve.

### 2.4.2 Radical ABTS scavenging assay

Both RSL and RSR extracts’ antioxidative activity were also investigated using a previously described method [18], with slight modification. Freshly prepared solutions of ABTS$^+$ (7 mM) and potassium persulfate (2.45 mM) were kept in the dark for 12 h, then mixed together, and incubated overnight in the refrigerator. The ABTS solution was diluted in ethanol, and different concentrations of RSLs and RSRs were allowed to react with ABTS solution (1:1). Absorbance was measured at wavelength ($\lambda$) 734 nm. The scavenging capacity of both extracts was compared to that of ascorbic acid, the standard antioxidant. The percentage of antioxidant capacity was calculated as described earlier [19], as follows:

\[
\% \text{ of radical scavenging activity} = \frac{(\text{Abs control} - \text{Abs sample}/\text{Abs control}) \times 100.}
\]

### 2.5 Cytotoxic activity

The effect of leaf and root extracts on the proliferation and viability of two breasts (MCF-7 and MDA-MB-231), liver (HepG2), and lung (A549) cancer cells were evaluated using the MTT colorimetric, according to a previously described protocol [20]. Briefly, 1 mL of the cell suspension was dispensed into a 24-well plate at a density of $5 \times 10^4$ cells/well in triplicate. Cells were then incubated for 24 h at 37°C and 5% CO$_2$ before treatment with different concentrations (500, 250, 125, and 62.5 µg/mL) of both RSLs and RSRs for 48 h. Doxorubicin-treated cells and the untreated cells were used as the positive and negative controls, respectively. After the incubation period, 100 µL of MTT solution (5 mg/mL) was added and further incubated for 4 h. Thereafter, formazan product was solubilized by acidified isopropanol and the optical density was read at 570 nm using a microplate reader (Bio-Tek, USA). The percentage of viable cells was computed using the formula:

\[
\text{Cell viability} (%) = \frac{(\text{O.D of the treated sample})}{(\text{O.D of the untreated sample})} \times 100%.
\]

### 2.6 HPTLC analysis

The HPTLC analysis of rutin present in RSL and RSR extracts was performed on the RP-HPTLC plate (10 × 10 cm). The dimensions of the band for each track were 0.6 and 0.94 cm apart. The speed (160 nL/s) was applied on the RP-HPTLC plate for both extracts as well as rutin compound. Pre-saturated twin-trough glass chamber (10 × 10 cm) at 25 ± 2°C under 60 ± 5% humidity was used for plate applied development. The developed plate of RP-HPTLC was dehydrated and quantitatively evaluated in the absorbance mode at $\lambda = 254$ nm.

### 2.7 Preparation of stock and standard solutions

The standard stock solution of rutin biomarker (PhytoLab, Vestenbergsgreuth, Germany) was prepared with methanol (HPLC grade) at a final concentration of 1 mg/mL. Next seven different dilutions ranging from 2 to 14 µg/mL were prepared with methanol using the rutin standard stock solution. All the dilutions were applied on the RP-HPTLC plate through a microliter syringe linked to the applicator to enhance the range of linearity (200–1,400 ng/band).

### 2.8 Statistical analysis

The data values of the untreated and treated samples of two parameters were analyzed using student’s $t$-test with excel Microsoft office. Data represent the mean ± S.D., and the difference was statistically significant at $^*P < 0.05$ and $^{**}P < 0.01$ compared to the control. The IC$_{50}$ values were calculated by probit analysis using OriginPro 8.5 software.

**Ethical approval:** The conducted research is not related to either human or animal use.
3 Results and discussion

3.1 Contents of total phenols and flavonoids

Both RSLs and RSRs exhibited different values of TPC and TFC contents. As schemed in Figure 1, RSLs exhibited a higher TPC (125.3 mg of GAE/g) compared with RSRs (95.8 of GAE/g). The value of TPC reported here for radish root was higher than the value (91.8 mg GAE/g) previously reported by Eugenio et al. [21]. Additionally, we found that the TPC of radish leaves was higher than that of the roots. This variance in TPC between different parts could be due to several factors that interfere with polyphenol biosynthesis, including postharvest handling, intra-specific chemodiversity, plant breeding, and environmental factors [22]. Regarding flavonoids, we found that the TFC in leaves was nearly two times higher than that in the roots (44.5 vs 24.4 of mg of QE/g) (Figure 1). Flavonoids constitute a special class of phenolic compounds that contain multiple hydroxyl groups, which confer valuable antioxidant activities to these phytoconstituents [23]. The protective effects of foods rich in flavonoids and phenolic compounds against several chronic ailments, including cardiovascular and cancer, have been well-documented [24,25]. Our results are consistent with those published by Goyeneche et al. who reported that phenols and flavonoids were most abundant in red radish leaves compared with its roots [26]. The beneficial effects of phenols and flavonoids may be associated with several activities they exert, the most important being the antioxidative effect [27,28]. Therefore, we next examined the antioxidative properties of both RSLs and RSRs.

3.2 Antioxidant activity

RSLs and RSRs showed dose-dependent scavenging activities (Table 1). RSL showed stronger antioxidant activity than RSR (IC50 216.8 vs 359.7 µg/mL) in the DPPH scavenging activity test. On the other hand, both RSLs and RSRs exerted dose-dependent antioxidative activity (Figure 2), and the calculated RSL IC50 was 326.7 µg/mL and RSR IC50 was 549 µg/mL in the ABTS test.

Due to their critical role in reducing oxidative stress, natural antioxidants play a vital role in human health [29].

Table 1: Scavenging activities of RSL and RSR extracts

| Sample        | 10  | 50  | 100 | 500 | 1,000 |
|---------------|-----|-----|-----|-----|-------|
|               | (µg/mL) | (µg/mL) | (µg/mL) | (µg/mL) | (µg/mL) |
| (%) DPPH radical scavenging activity |       |       |       |       |       |
| RSL           | 13.7 ± 0.7 | 23.1 ± 1.1 | 46.5 ± 1.4 | 58.1 ± 1.5 | 72 ± 1.6 |
| RSR           | 12.4 ± 3.1 | 19.8 ± 1.3 | 40.6 ± 2 | 55 ± 1.7 | 67.9 ± 0.3 |
| Ascorbic acid | 82.6 ± 2.1 | 85.7 ± 1.2 | 86 ± 2.4 | 89.6 ± 1.6 | 91.2 ± 1.5 |
| (%) ABTS radical cation scavenging activity |       |       |       |       |       |
| RSL           | 12.2 ± 3.7 | 20.6 ± 3.2 | 44.2 ± 2.6 | 54.4 ± 1.4 | 71.4 ± 0.6 |
| RSR           | 11.4 ± 0.6 | 17.1 ± 1.4 | 39.7 ± 0.4 | 48.4 ± 1.7 | 65 ± 1.6 |
| Ascorbic acid | 81.7 ± 1.4 | 84.1 ± 2.1 | 85.4 ± 2.4 | 88.2 ± 2.3 | 89.8 ± 2.1 |

Figure 2: Antioxidant scavenging activities of R. sativus leaves and roots (DPPH and ABTS).
The type of antioxidant assay is dependent on the characteristics of the specific system that can alter the analysis results. Therefore, one assay would not be representative of the antioxidant activity of plant extracts [30]. Hence, we used two different methods in the present study to examine the antioxidant activity of RSLs and RSRs, which could provide more reliable results. Both the RSLs and RSRs exhibited dose-dependent scavenging activities (Table 1) in both the DPPH and ABTS methods. We also found that the leaves demonstrated a stronger antioxidant activity than the roots. Our results were consistent with the study conducted by Agarwal and Varma [31] who found that the antioxidant activity of RSL extracts was more potent than that of the root extract. Similar results were reported by Chorol who investigated the phenolic and antioxidant profiles of four different radish parts and found that the leaves and peels demonstrated the most considerable amount of antioxidant activity [32]. Polyphenolics’ presence with their well-known radical scavenging properties can explain this previously documented activity in different model systems [33]. Moreover, our results support the positive correlation between the phenolic content and the reducing capacity or antioxidant activities of plant extracts, as previously reported [34–36]. Besides, it has been reported that the RSLs are a rich source of antioxidant compounds [37], which confirms our study results.

### 3.3 Anticancer activity

For investigating the anticancer potential of plant extracts, it is important to clearly explore the different pharmacological uses of those plants [38]. Previous research has explored the anticancer properties of different parts of *R. sativus* using various cancer cells [39]. In the present study, the MTT assay results revealed that both RSLs and RSRs exhibited antiproliferative activity against all the tested cancer cells. The viability of all the treated cells decreased in a dose-dependent manner (Figure 3). Based on the IC50 value (half-inhibitory concentration) of leaves and roots, it was found that the leaf extract has a stronger anticancer activity than the root extract (Table 2).

Consistent with our study results, Kim et al. also observed that the ethanolic leaf extract of *R. sativus* significantly decreased MDA-MB-231 breast cancer cells’ proliferation after 48 h incubation [40]. The antiproliferative effects of different radish parts were also evaluated in different cancer cells by Beevi et al. They found that the root hexane extract of *R. sativus* inhibited the

### Table 2: IC50 values of RSLs and RSRs against different cancer cells

| Extract | Cell lines and IC50 (µg/mL) |
|---------|-----------------------------|
|         | A549 | HepG2 | MCF-7 | MDA-MB-231 |
| RSLs    | 217 ± 2.1 | 287 ± 2.3 | 224 ± 2.2 | 453.2 ± 1.9 |
| RSRs    | 250.6 ± 3.1 | 444.6 ± 1.6 | 306.3 ± 2.2 | 470 ± 2.6 |
| Doxorubicin | 1 ± 0.02 | 1.4 ± 0.04 | 1.2 ± 0.03 | 1.1 ± 0.04 |
proliferation of different cancer cell lines, including A549 and MCF-7 cells, through the induction of apoptosis [41]. This antiproliferative activity could be attributed to the presence of several bioactive constituents in R. sativus, such as 4-methylthio-butanyl derivatives [42,43], sulforaphene [44], and different flavonoid compounds [45], which were found to exhibit antitumor activities against different cancer cells. Moreover, the compound rutin, quantified in this study, is known for its anticancer effect in several cancer cell lines [46–50].

4 HPTLC analysis

4.1 Rutin identification

The HPTLC analysis of the RSL and RSR extracts demonstrated the presence of rutin in the leaf extract, whereas rutin peak was not detected in the root extract (Figure 4).

4.2 Concurrent analysis of rutin in the methanolic extract of RSLs by HPTLC method

The suitable mobile phase for the quantitative analysis of rutin in RSR extract using HPTLC was selected by examining the various compositions of different solvents, and a combination of toluene, ethyl acetate, and formic acid (7:3:0.1; v/v/v) was found to be the most suitable mobile phase (Table 3). This method resulted in intense peaks of rutin at Rf = 0.65 and 0.67 and very clearly separated the standards and the different phytoconstituents of RSR extract (Figure 5). The regression equation (y)/correlation coefficient (r²) for rutin was found to be 5.899x + 1161/0.9978 (Table 3). The HPTLC method was used to analyze rutin concurrently in the RSL extract, which revealed a rutin content of 5.2 mg/g of the extract’s dry weight.

Rutin (Figure 6) is a polyphenolic bioflavonoid compound and one of the active constituents found in different parts of several plant species. Various studies have demonstrated its broad nutraceutical and pharmacological effects in treating complex chronic diseases such as cancer, cardiovascular disorders, and diabetes [48,51]. The growing area’s source and geographical conditions play a vital role in determining the components and activities of plant extracts [52]. Therefore, we developed

Table 3: Linear regression and Rf data, for rutin calibration curve

| Parameters                  | Rutin          |
|-----------------------------|----------------|
| Linearity range (ng/spot)   | 200–1,400      |
| Regression equation         | y = 4.9267x − 736.25 |
| Correlation coefficient (r²)| 0.9971         |
| Slope                       | 4.926721429    |
| Intercept                   | 736.2          |
| Rf value                    | 0.65           |
| LOD (ng)                    | 71.68          |
| LOQ (ng)                    | 217.22         |

Rf: retention factor, LOD: limit of detection, LOQ: limit of quantitation.
a simple, optimized, and validated method in the current study to standardize rutin in *R. sativus* grown in Saudi Arabia using HPTLC. The HPTLC method may serve as an alternative to HPLC or LC-MS/MS, specifically in analyzing crude plant extracts. In contrast to HPLC, a quantitative analysis conducted using HPTLC does not require extensive clean-up procedures. Moreover, the availability of numerous accurate reagents for the confirmation and detection of specific compounds is considered as a valuable advantage of HPTLC [53,54].

5 Conclusion

Plant products have been receiving increasing research attention as alternative medicine. This current study demonstrated that the leaves of *R. sativus* grown in Saudi Arabia consisted of the maximum phenol and flavonoid constituents and demonstrated the highest free radical scavenging capacity than the roots. The antioxidant biomarker compound rutin was detected only in radish leaves, as revealed by the RP-HPTLC method developed in this work. Altogether, our study results showed that radish leaves could be a better source of bioactive compounds, especially flavonoids than roots. Therefore, the inclusion of these leaves in the human diet could increase flavonoid compounds’ intake due to their high level. In conclusion, radish leaves and roots’ consumption may confer a nutritional, medicinal value to human health. Furthermore, extensive research is needed to optimize and estimate other compounds from the leaves to develop novel functional products.

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