# **Research Article**

Omar M. Noman\*, Fahd A. Nasr\*, Ali S. Alqahtani, Mohammed Al-zharani, Mary Anne W. Cordero, Amal A. Alotaibi, Asmatanzeem Bepari, Saud Alarifi, Ali Daoud

# 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

https://doi.org/10.1515/chem-2021-0042 received December 1, 2020; accepted March 5, 2021

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) were 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 (IC50 216.8 vs 359.7  $\mu$ g/mL) and ABTS (IC50 326.7 vs 549  $\mu$ g/mL) models. RSLs also demonstrated the highest antiproliferative efficiency against all cell lines, with IC50 values of 217–453  $\mu$ g/mL. The reversed-phase-high-performance thin-layer chromatography results showed the presence of rutin (5.2  $\mu$ g/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], antimutagenic [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].

Mohammed Al-zharani: Biology Department, College of Science, Imam Mohammad ibn Saud Islamic University (IMSIU), Riyadh 11623, Saudi Arabia

Mary Anne W. Cordero, Amal A. Alotaibi, Asmatanzeem Bepari:
Basic Science Department, College of Medicine, Princess Nourah
bint Abdulrahman University, Riyadh 11671, Saudi Arabia
Saud Alarifi, Ali Daoud: Department of Zoology, College of Science,
King Saud University, Riyadh 11451, Saudi Arabia

<sup>\*</sup> Corresponding author: Omar M. Noman, Medicinal Aromatic and Poisonous Plants Research Centre, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia, e-mail: onoman@ksu.edu.sa

<sup>\*</sup> Corresponding author: Fahd A. Nasr, Medicinal Aromatic and Poisonous Plants Research Centre, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia, e-mail: fnasr@ksu.edu.sa Ali S. Alqahtani: Medicinal Aromatic and Poisonous Plants Research Centre, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

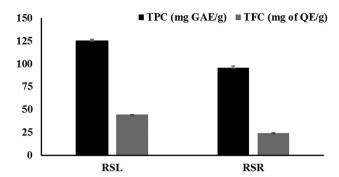


Figure 1: Total contents of phenols and flavonoids in RSLs and RSRs.

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.

# 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  $\pm$  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,  $100\,\mu L$  of  $0.5\,N$  Folin–Ciocalteu reagent was added to  $500\,\mu L$  of each extract. This was followed by a thorough mixing of all contents within the flask, after which  $2.5\,m L$  sodium carbonate was included. The mixture was incubated for  $0.5\,h$ . Finally, the phenolic contents' absorbance was measured at  $760\,n m$  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% AlCl<sub>3</sub> 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  $\mu$ g/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  $\lambda_{max}=517\, nm$ , and ascorbic acid served as a positive control. The radical scavenging activity was computed using the given formula:

% of radical scavenging activity

= (Abs control - Abs extract/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<sup>+</sup> (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:

% of radical scavenging activity

= (Abs control - Abs sample/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<sub>2</sub> 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:

Cell viability (%) = (0.D of the treated sample) /(0.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  $\times$  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  $\times$  10 cm) at 25  $\pm$  2°C under 60  $\pm$  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  $14 \,\mu g/mL$  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  $\pm$  S.D., and the difference was statistically significant at  $^*P < 0.05$  and  $^{**}P < 0.01$  compared to the control. The IC<sub>50</sub> 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.

Table 1: Scavenging activities of RSL and RSR extracts

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

#### 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, intraspecific 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 (IC $_{50}$  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 IC $_{50}$  was 326.7 µg/mL and RSR IC $_{50}$  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].

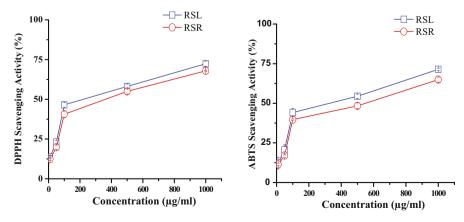


Figure 2: Antioxidant scavenging activities of R. sativus leaves and roots (DPPH and ABTS).

412 — Omar M. Noman et al. DE GRUYTER

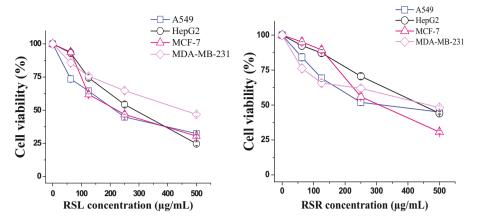


Figure 3: R. sativus leaf and root extracts inhibited the proliferation of various cancer cells. Cells were plated as described in materials, and MTT assay was performed to explore the inhibitory effects of RSLs and RSRs on cancer cell proliferation after 48 h of incubation. Data represent the mean  $\pm$  SD of three independent experiments.

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.

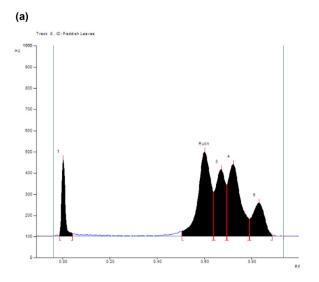
#### 3.3 Anticancer activity

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  $IC_{50}$  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 IC <sub>50</sub> (μg/mL)				
	A549	HepG2	MCF-7	MDA-MB-231	
RSLs	217 ± 2.1	287 ± 2.3	224 ± 2.2	453.2 ± 1.9	
RSRs	$250.6 \pm 3.1$	$444.6 \pm 1.6$	$306.3 \pm 2.2$	$470 \pm 2.6$	
Doxorubicin	$1\pm0.02$	$1.4\pm0.04$	$1.2\pm0.03$	$\textbf{1.1} \pm \textbf{0.04}$	



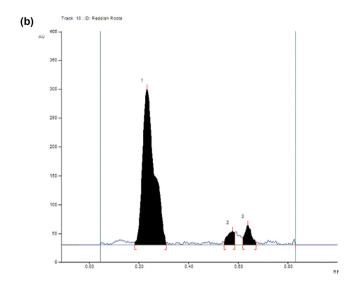


Figure 4: Identification of rutin in RSL and RSR extracts. Rutin was detected in RSLs (left), whereas it was absent in RSR extract (right). (a) HPTLC chromatogram of RSLs containing rutin, (b) HPTLC chromatogram of RSLs not containing rutin.

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

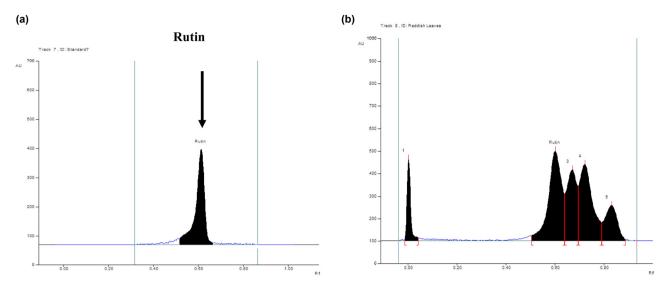
phase (Table 3). This method resulted in intense peaks of rutin at  $R_f = 0.65$  and 0.67 and very clearly separated the standards and the different phytoconstituents of RSR extract (Figure 5). The regression equation (y)/correlation coefficient  $(r^2)$  for rutin was found to be 5.899x + 1161/ 0.9978 (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  $R_f$  data, for rutin calibration curve

Parameters	Rutin		
Linearity range (ng/spot)	200-1,400		
Regression equation	y = 4.9267x - 736.25		
Correlation coefficient $(r^2)$	0.9971		
Slope	4.926721429		
Intercept	736.2		
R <sub>f</sub> value	0.65		
LOD (ng)	71.68		
LOQ (ng)	217.22		

Rf: retention factor, LOD: limit of detection, LOQ: limit of quantitation.



**Figure 5:** Quantification of rutin in RSL by RP-HPTLC. (a) Chromatogram of standard rutin ( $R_f = 0.65$ ; 800 ng/spot) at 365 nm (left). (b) Chromatogram of RSL extract (Rutin, spot 2,  $R_f = 0.65$ ); [mobile phase: acetonitrile:water, (3.5:6.5, v/v)].

Figure 6: Chemical structure of the biomarker rutin.

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.

**Acknowledgments:** This work was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University, through the Research Groups Program Grant no. (RGP-1442-0033).

**Author contributions:** Conceptualization, A. S. A; methodology, O. M. N., F. A. N,. and M. Z.; validation, S. A. and A. D.; data curation, writing – original draft preparation, O. M. N., A. A. A., and M. A. W. C.; writing – review and editing, A. A. A., O. M. N., A. S. A., and A. B., funding acquisition, M. A. W. C. and A. B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University, through the Research Groups Program Grant no. (RGP-1442-0033).

**Competing interest:** The authors declare that they have no competing interests.

Data availability statement: The data sets used and/or analyzed during the current study are available with the corresponding author on reasonable request.

## References

- Aruna G, Yerragunt VG, Raju AB. Photochemistry and pharmacology of Raphanus sativus. Int J Drug Formulation Res. 2012;3(1):43-52.
- Beevi SS, Mangamoori LN, Anabrolu N. Comparative activity against pathogenic bacteria of the root, stem, and leaf of Raphanus sativus grown in India. World J Microbiol Biotechnol. 2009;25(3):465-73.
- Manchali S, Murthy KNC, Patil BS. Crucial facts about health benefits of popular cruciferous vegetables. J Funct Foods. 2012;4(1):94-106.
- Ahmad F, Hasan I, Chishti DK, Ahmad H. Antibacterial activity of Raphanus sativus Linn. seed extract. Glob J Med Res. 2012;12(11):25-33.
- Lugasi A, Dworschák E, Blazovics A, Kery A. Antioxidant and free radical scavenging properties of squeezed juice from black radish (Raphanus sativus L. var niger) root. Phytother Res. 1998;12(7):502-6.
- Nakamura Y, Iwahashi T, Tanaka A, Koutani J, Matsuo T, Okamoto S, et al. 4-(Methylthio)-3-butenyl isothiocyanate, a principal antimutagen in daikon (Raphanus sativus; Japanese white radish). J Agric Food Chem. 2001;49(12):5755-60.
- Hecht SS, Kenney PM, Wang M, Trushin N, Upadhyaya P. Effects of phenethyl isothiocyanate and benzyl isothiocyanate, individually and in combination, on lung tumorigenesis induced in A/I mice by benzo [a] pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Lett. 2000;150(1):49-56.
- [8] Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 2001;56(1):5-51.
- [9] Gutiérrez RMP, Perez RL. Raphanus sativus (radish): their chemistry and biology. Sci World J. 2004;4:811-37.
- [10] Montaut S, Barillari J, Iori R, Rollin P. Glucoraphasatin: chemistry, occurrence, and biological properties. Phytochemistry. 2010;71(1):6-12.
- Kim KH, Kim CS, Park YJ, Moon E, Choi SU, Lee JH, et al. Antiinflammatory and antitumor phenylpropanoid sucrosides from the seeds of Raphanus sativus. Bioorg Med Chem Lett. 2015;25(1):96-9.
- [12] Shin T, Ahn M, Kim GO, Park SU. Biological activity of various radish species. Orient Pharm Exp Med. 2015;15(2):105-11.
- [13] Devaraj VC, Krishna B, Viswanatha GL. Simultaneous determination of quercetin, rutin and kaempferol in the leaf extracts of Moringa oleifera Lam. and Raphinus sativus Linn. by liquid chromatography-tandem mass spectrometry. J Chin Integr Med. 2011;9(9):1022-30.

- [14] Ngoc PTK, Nguyet NTM, Dao DTA. In antimicrobial and antioxidant properties of the flavonoid extract from Raphanus sativus L. AIP conference proceedings. AIP Publishing LLC; 2017. p. 020026.
- [15] Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Vitic. 1977;28(1):49-55.
- [16] Mervat M, Far E, Hanan A, Taie A. Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. Aust J Basic Appl Sci. 2009;3(4):3609-16.
- [17] Brand-Williams W, Cuvelier M-E, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995;28(1):25-30.
- [18] Li W, Hosseinian FS, Tsopmo A, Friel JK, Beta T. Evaluation of antioxidant capacity and aroma quality of breast milk. Nutrition. 2009;25(1):105-14.
- [19] Celli GB, Pereira-Netto AB, Beta T. Comparative analysis of total phenolic content, antioxidant activity, and flavonoids profile of fruits from two varieties of Brazilian cherry (Eugenia uniflora L.) throughout the fruit developmental stages. Food Res Int. 2011;44(8):2442-51.
- [20] Al-Zharani M, Nasr FA, Abutaha N, Alqahtani AS, Noman OM, Mubarak M, et al. Apoptotic induction and anti-migratory effects of rhazya stricta fruit extracts on a human breast cancer cell line. Molecules (Basel, Switzerland). 2019;24(21):3968.
- [21] Eugenio MHA, Pereira RGFA, Abreu WCd, Pereira MCd. A. Phenolic compounds and antioxidant activity of tuberous root leaves. Int J Food Prop. 2017;20(12):2966-73.
- [22] Bruni R, Sacchetti G. Factors affecting polyphenol biosynthesis in wild and field grown St. John's Wort (Hypericum perforatum L. hypericaceae/guttiferae). Molecules. 2009;14(2):682-725.
- [23] Kim J-S, Kang O-J, Gweon O-C. Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. J Funct Foods. 2013;5(1):80-6.
- Del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxid Redox Signal. 2013;18(14):1818-92.
- [25] Rodriguez-Mateos A, Vauzour D, Krueger CG, Shanmuganayagam D, Reed J, Calani L, et al. Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. Arch Toxicol. 2014;88(10):1803-53.
- Goyeneche R, Roura S, Ponce A, Vega-Gálvez A, Quispe-Fuentes I, Uribe E, et al. Chemical characterization and antioxidant capacity of red radish (Raphanus sativus L.) leaves and roots. J Funct Foods. 2015;16:256-64.
- [27] Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997;2(4):152-9.
- Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47.
- [29] Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev. 2010;4(8):118-26.
- [30] Moon JK, Shibamoto T. Antioxidant assays for plant and food components. J Agric Food Chem. 2009;57(5):1655-66.

- [31] Agarwal K, Varma R. Radical scavenging ability and biochemical screening of a common asian vegetable *Raphanus* sativus L. Int J Pharm Sci Rev Res. 2014;27(1):127–34.
- [32] Chorol S. Antioxidant content in different parts of radish (*Raphanus sativus* L.) from cold arid Ladakh region of trans-Himalaya (Jammu and Kashmir). Pharmacogn J. 2019;11:5.
- [33] Beevi SS, Mangamoori LN, Gowda BB. Polyphenolics profile and antioxidant properties of *Raphanus sativus* L. Nat Product Res. 2012;26(6):557-63.
- [34] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2:270-8.
- [35] Piluzza G, Bullitta S. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. Pharm Biol. 2011;49(3):240–7.
- [36] Terpinc P, Čeh B, Ulrih NP, Abramovič H. Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts. Ind Crop Prod. 2012;39:210-7.
- [37] Reddy PV, Desai S, Ahmed F, Urooj A. Antioxidant properties and stability of *Raphanus sativus* extracts. J Pharm Res. 2011;3(3):658-61.
- [38] Greenwell M, Rahman PKSM. Medicinal plants: their use in anticancer treatment. Int J Pharm Sci Res. 2015;6(10):4103–12.
- [39] Manivannan A, Kim J-H, Kim D-S, Lee E-S, Lee H-E. Deciphering the nutraceutical potential of *Raphanus sativus* - A comprehensive overview. Nutrients. 2019;11(2):402.
- [40] Kim WK, Kim JH, Jeong DH, Chun YH, Kim SH, Cho KJ, et al. Radish (*Raphanus sativus* L. leaf) ethanol extract inhibits protein and mRNA expression of ErbB(2) and ErbB(3) in MDA-MB-231 human breast cancer cells. Nutr Res Pract. 2011;5(4):288-93.
- [41] Beevi SS, Mangamoori LN, Subathra M, Edula JR. Hexane extract of *Raphanus sativus* L. roots inhibits cell proliferation and induces apoptosis in human cancer cells by modulating genes related to apoptotic pathway. Plant Foods Hum Nutr. 2010;65(3):200–9.
- [42] Papi A, Orlandi M, Bartolini G, Barillari J, Iori R, Paolini M, et al. Cytotoxic and antioxidant activity of 4-methylthio-3-butenyl isothiocyanate from *Raphanus sativus* L. (Kaiware Daikon) sprouts. J Agric Food Chem. 2008;56(3):875–83.
- [43] Kim KH, Moon E, Kim SY, Choi SU, Lee JH, Lee KR. 4-Methylthio-butanyl derivatives from the seeds of *Raphanus* sativus and their biological evaluation on anti-inflammatory

- and antitumor activities. J Ethnopharmacol. 2014;151(1):503-8.
- [44] Pawlik A, Wała M, Hać A, Felczykowska A, Herman-Antosiewicz A. Sulforaphene, an isothiocyanate present in radish plants, inhibits proliferation of human breast cancer cells. Phytomedicine. 2017;29:1–10.
- [45] Sangthong S, Weerapreeyakul N, Lehtonen M, Leppanen J, Rautio J. High-accuracy mass spectrometry for identification of sulphur-containing bioactive constituents and flavonoids in extracts of *Raphanus sativus* var. caudatus Alef (Thai rat-tailed radish). J Funct Foods. 2017;31:237–47.
- [46] Chen H, Miao Q, Geng M, Liu J, Hu Y, Tian L, et al. Anti-tumor effect of rutin on human neuroblastoma cell lines through inducing G2/M cell cycle arrest and promoting apoptosis. Sci World J. 2013;2013:269165.
- [47] Perk AA, Shatynska-Mytsyk I, Gerçek YC, Boztaş K, Yazgan M, Fayyaz S, et al. Rutin mediated targeting of signaling machinery in cancer cells. Cancer Cell Int. 2014;14(1):1-5.
- [48] Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. Saudi Pharm J. 2017;25(2):149-64.
- [49] Gullón B, Lú-Chau TA, Moreira MT, Lema JM, Eibes G. Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. Trends Food Sci Technol. 2017;67:220–35.
- [50] Caparica R, Júlio A, Araújo MEM, Baby AR, Fonte P, Costa JG, et al. Anticancer activity of rutin and its combination with ionic liquids on renal cells. Biomolecules. 2020;10(2):233.
- [51] Sharma S, Ali A, Ali J, Sahni JK, Baboota S. Rutin: therapeutic potential and recent advances in drug delivery. Expert Opin Invest Drugs. 2013;22(8):1063-79.
- [52] Schaffer S, Schmitt-Schillig S, Müller WE, Eckert GP.
  Antioxidant properties of Mediterranean food plant extracts: geographical differences. J Physiol Pharmacol. 2005;56(Suppl 1):115-24.
- [53] Wójciak-Kosior M, Skalska A, Matysik A. Determination of phenothiazine derivatives by high performance thin-layer chromatography combined with densitometry. J Pharm Biomed Anal. 2006;41(1):286-9.
- [54] Vilegas JH, Lanças FM, Wauters JN, Angenot L. Characterization of adulteration of "espinheira santa" (Maytenus ilicifolia and Maytenus aquifolium, Celastraceae) hydroalcoholic extracts with Sorocea bomplandii (Moraceae) by high-performance thin layer chromatography. Phytochem Anal. 1998;9(6):263–6.