Pharmacogn J. 2022; 14(4): 313-318
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Insight into Screening of Secondary Metabolites, Phenolic and Flavonoid Contents and Antioxidant Activity of *Raphanus sativus* L. Cultivated in Eastern Province of Saudi Arabia

Abdullah Abdulhamid Altaweel*, Abdullah Jalal Alasoom, Hussein Ali Burshed, Marwan Mohamed Alshawush, Hany Ezzat Khalil*

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 (0.036±0.03) and (11.57±0.60) mg QE/g extract for leaves and roots extracts 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 IC_{50}: (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.

Abdullah Abdulhamid Altaweel*, Abdullah Jalal Alasoom, Hussein Ali Burshed, Marwan Mohamed Alshawush, Hany Ezzat Khalil*

Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa 31982, SAUDI ARABIA.

Correspondence

Abdullah Abdulhamid Altaweel

Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa 31982, SAUDI ARABIA.

E-mail: 220005586@student.kfu.edu.sa

Hany Ezzat Khalil

Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa 31982, SAUDI ARABIA.

E-mail: heahmed@kfu.edu.sa

History

- Submission Date: 03-05-2022;
- Review completed: 03-06-2022;
- Accepted Date: 08-06-2022.

DOI : 10.5530/pj.2022.14.101

Article Available online

http://www.phcogj.com/v14/i3

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.[1-3] Obviously, 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.[4] However, synthetic antioxidants are available to help but they may have toxic and adverse effects [5] 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.[6] 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.[7-9] *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.[10] 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.[11-14] 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.[15] 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

Cite this article: Altaweel AA, Alasoom AJ, Burshed HA, Alshawush MM, Khalil HE. Insight 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.

Arabia. Leaves and roots were separated and subjected to air-drying according to standard herbarium procedures. A voucher sample (RS-2021) was kept in Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University. All reagents and chemicals were of the finest grade available

Extraction

The air-dried powdered leaves and roots (300 g and 200 g, respectively) were thoroughly extracted three times (for 5 days) using 3 L of 70% methanol (Sigma Aldrich, Germany), applying cold maceration method to avoid destruction of secondary metabolites Various extracts were concentrated using rotovap and then freeze-dried to yield the total dry extracts of leaves and roots, 24 g and 10 g respectively, which were kept in freezer for the next steps. The total extracts of leaves and roots, (20 g and 7 g, respectively) were mixed with distilled water (500 mL) and fractionated with n-hexane (4×500 ml). The n-hexane fractions were combined and concentrated to yield 9.5 g and 1.3 g, for leaves and roots respectively. The remaining aqueous fraction was subjected to further fractionation with chloroform (4×500 mL). The chloroform fractions were collected and concentrated to give 3.5 g and 1.2 g, for leaves and roots respectively. Similarly, the ethyl acetate fraction was developed to give to give 4.3 g and 2.4 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

Qualitative screening of the secondary metabolites was performed according to the standard protocols.[16,17]

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 diluted acid. This result indicates the presence of flavonoid.

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 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.

Anthraquinones

Extract 0.5 ml was boiled with dilute sulphuric acid then filtered and cooled. The filtrate was extracted with chloroform and dilute ammonia solution was added to it. The aqueous ammonia layer becomes pink to red due to the presence of anthraquinones derivatives.

Cardiac glycosides

Extract of 10 ml was mixed with (4 ml) of solution of glacial acetic acid and 1 drop of 2% ferric chloride followed by 1 ml of concentrated sulphuric acid. A brown ring formed between the layers demonstrates the presence of cardiac glycosides.

Carbohydrates

Plant extract of 2 ml was mixed with a 10 ml Molisch reagent. Then, 2 ml concentrated sulphuric acid was added from the side of the test tube. The formation of a violet ring at the junction of two phases indicates the presence of carbohydrates.

Fourier transform infrared spectroscopy (FTIR) characterization

Characterization of various functional groups of various phyto-constituents was inspected employing FTIR using FTIR spectrophotometer by means of potassium bromide pellet technique. Potassium bromide discs were developed and about 5µL of different fractions were dropped on the discs and dried. The prepared discs of different fractions were inspected for FTIR (spectra ranged between 4000 and 400 cm^{-1}).[18]

Determination of total phenolic content (TPC)

TPC was evaluated using the Folin-Ciocalteu index protocol.[19] Stock solutions with a concentration of 1 mg/ml of different fractions were arranged in methanol. Folin-Ciocalteu reagent (0.5 ml) and 6 ml of double distilled deionized water were sequentially added to 0.1 ml of stock solution of each fraction. Later, 1.5 ml of a 20% sodium carbonate solution was added to obtain final volume of 10 ml. A reaction was completed for 2 h at 25°C Then, the absorbance of different mixtures was measured at 760 nm. Calibration curve of gallic acid (standard was prepared using serial dilution (0.5, 0.4, 0.3, 0.2 and 0.1 mg/ml in distilled water).

Determination of total flavonoid content (TFC)

TFC was investigated in accordance to Khalil et al method.[20] various fractions (10 mg) were diluted in (100 ml) of mixture with ratio of (1:1 v/v) consisting of deionized water and acetone. A solution of 0.25 ml of the serially diluted sample was added to 0.75 µl of a sodium nitrite (5% w/v) solution, followed by (0.15 ml) of a freshly prepared 10% aluminum chloride solution. The previously developed mixture was mixed with 0.5 ml of 1 M sodium hydroxide solution. Then, the volume of developed medium was adjusted to 10 ml with deionized double distilled water. The subsequent developed mixtures were set aside for 5 min and the absorbance was observed at 510 nm against the same components lacking of the sample. Calibration curve was prepared using quercetin as reference (0.0, 0.1, 0.5, 1, 5 and 10 µg/ml).

Determination of antioxidant activity (DPPH assay)

This protocol is based primarily on the reduction of reagent (2,2-diphenyl-1-picrylhydrazyl (DPPH)) dissolved in methanol and in presence of a donated protons from available molecules such as free radical scavenger substance, led to formation of non-radical form DPPH-H. The reduction in DPPH radicals was monitored by the reduction in its absorbance at 515 nm caused by antioxidant molecules

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:

% of inhibition = {1 – [Ab sample - Ab blank] / [Ab control - Ab blank]} ×100

Where: Ab control is the absorbance of mixture (with DMSO and all other reactant without tested extracts). IC_{50} was recorded as the sample concentration that is essential to produce inhibition of DPPH radical to be formed by 50%.[21]

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 fractions 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 hydroxyflavonoids. 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).[22] These results also confirmed the results of phytochemical screening.

TPC assessment

TPC in various fractions was calculated using the regression equation: $y = 0.169x - 0.0053$, $R^2 = 0.9578$. The quantity of phenolic constituents was expressed as the equivalence of milligrams of standard Gallic acid per gram of dried plant extract (mg GAE/g) (Figure 2A) Results demonstrated that the quantity of TPC ranged from (8.92±1.01) to (211.80±1.57) mg GAE/g of dry extract. Ethyl acetate fractions expressed the highest percentage of TPC followed by the aqueous fractions. Chloroform fractions expressed the least amount while n. hexane fraction contains very less phenolic constituents (Figure 2B).

TFC assessment

TFC in various fractions 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 fractions in TFC then aqueous fractions. Chloroform fractions demonstrated the least amount of TFC compared to other fractions. On the other hand, n-hexane fractions hardly contained any flavonoid constituents (Figure 3B). The results demonstrated that leaves fractions are richer in contents compared to roots fractions.

Antioxidant activity (DPPH)

Besides, various fractions were inspected for their radical scavenger potential using the DPPH free radical scavenging assay (Figure 4). Ethyl acetate fraction showed marked scavenging activities (IC_{50}: 47.2±1.5 and 58.7±0.7 µg/ml, for leaves and roots fractions respectively), aqueous fraction showed moderate activity (IC_{50}: 56.3±1.3 and 69.7±1.8 µg/ml for leaves and roots fractions respectively) and n-hexane and chloroform fractions demonstrated much weaker potentials with IC_{50} above 100 µg/ml, all results were comparable to the standard trolox (IC_{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 fractions. Results could be credited to the higher TPC and TFC in these fractions. 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*.

Phyto-constituent/Fraction	LnH	LCH	LEA	LAQ	RnH	RCH	REA	RAQ
Flavonoids	-	+	++	+	-	+	++	+
Alkaloids	-	-	-	-	-	-	-	-
Saponins	++	+	+	+	++	+	+	+
Steroids	+	+	+	+	+	+	+	+
Tannins/Phenols	-	-	++	+	-	-	+	+
Anthraquinones.	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-
Glycosides	-	-	+	+	-	-	+	+
Carbohydrates	-	-	++	+++	-	-	++	+++

LnH; leaves n-hexane fraction, LCH; leaves chloroform fraction, LEA; leaves ethyl acetate fraction, LAQ; leaves remaining aqueous fraction, RnH; roots n-hexane fraction, RCH; roots chloroform fraction, REA; roots ethyl acetate fraction, RAQ; roots remaining aqueous fraction Presence in high amount (+++), presence in moderate amount (++), presence in little amount (+), absence (-).

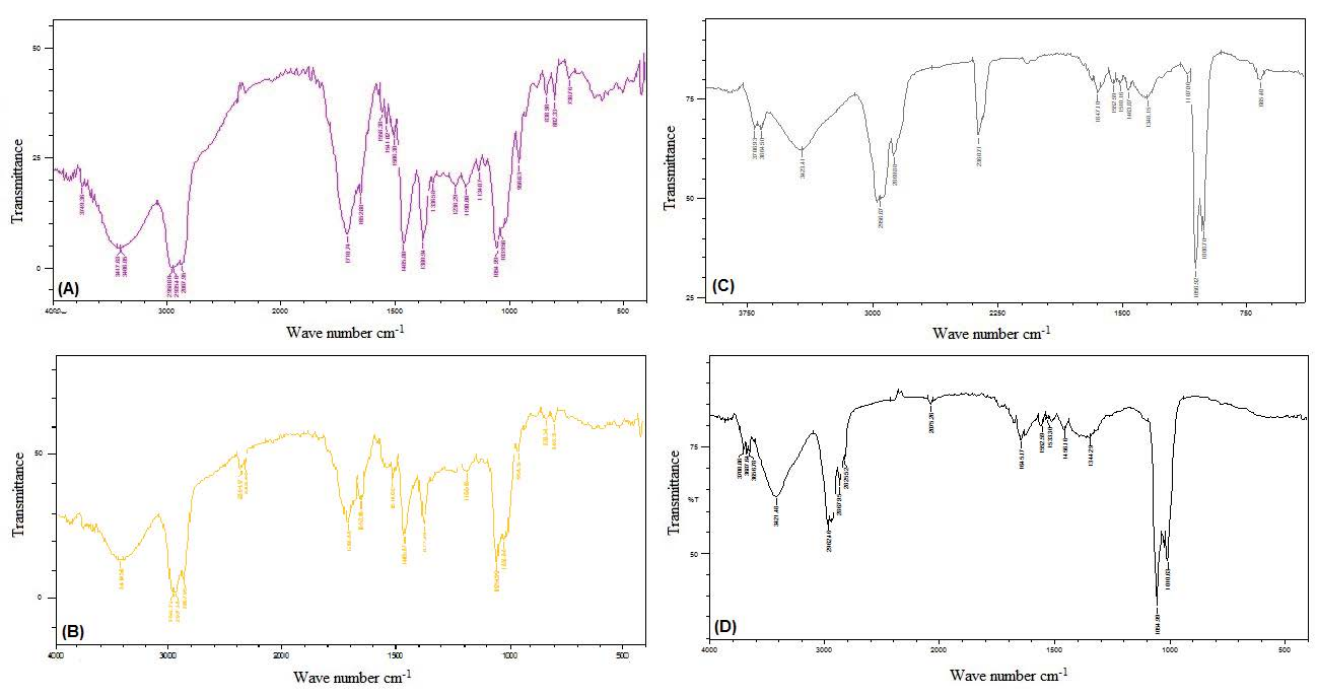


Figure 1: FTIR spectra of; ethyl acetate fraction of leaves (A), ethyl acetate fraction of roots (B), aqueous fraction of leaves (C) and aqueous fraction of roots (D).

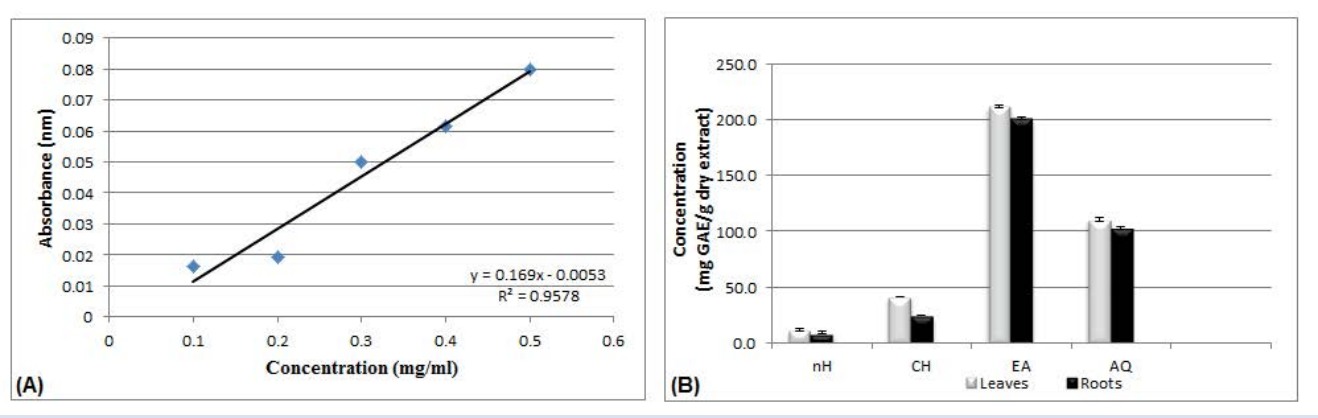


Figure 2: Calibration curve for gallic acid (A) and total phenolic contents (B) of various fractions of leaves and roots of *Raphanus sativus.* nH; n-hexane fraction, CH; chloroform fraction, EA; ethyl acetate fraction, AQ; remaining aqueous fraction, GAE/g; Gallic acid equivalence per gram of dried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

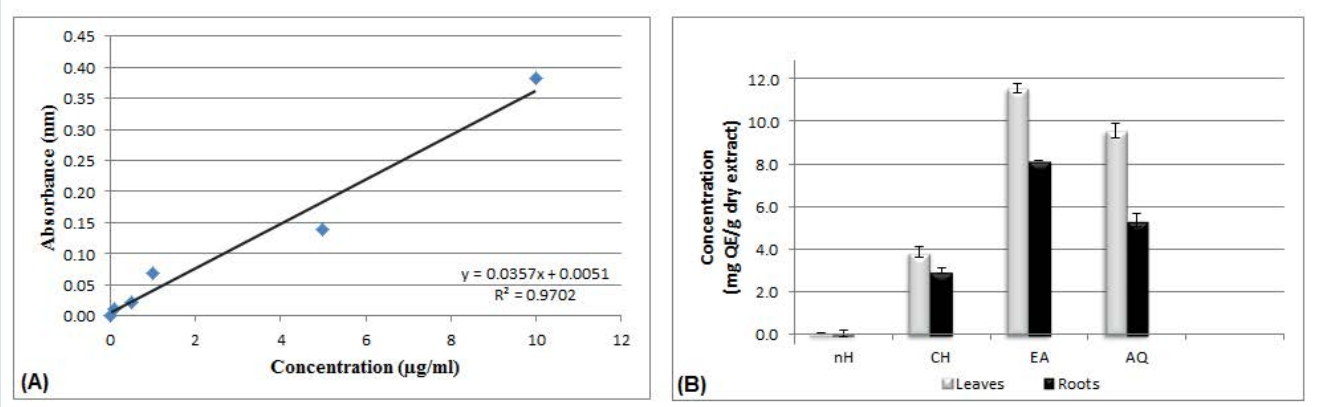


Figure 3: Calibration curve for quercetin (A) and total flavonoid contents (B) of various fractions of leaves and roots of *Raphanus sativus.* nH; n-hexane fraction, CH; chloroform fraction, EA; ethyl acetate fraction, AQ; remaining aqueous fraction, QE/g; quercetin equivalence per gram of dried plant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

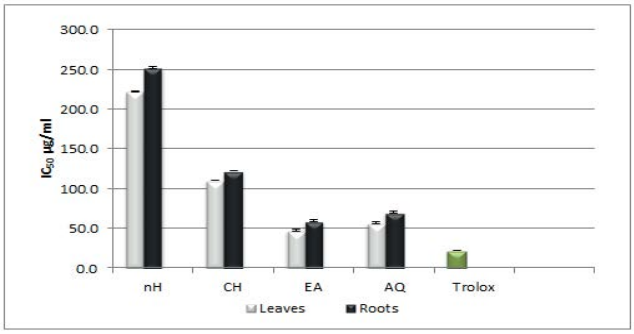


Figure 4: Antioxidant activity (DPPH) of various fractions of leaves and roots of *Raphanus sativus*. *nH*; n-hexane fraction, CH; chloroform fraction, EA; ethyl acetate fraction, AQ; remaining aqueous fraction.

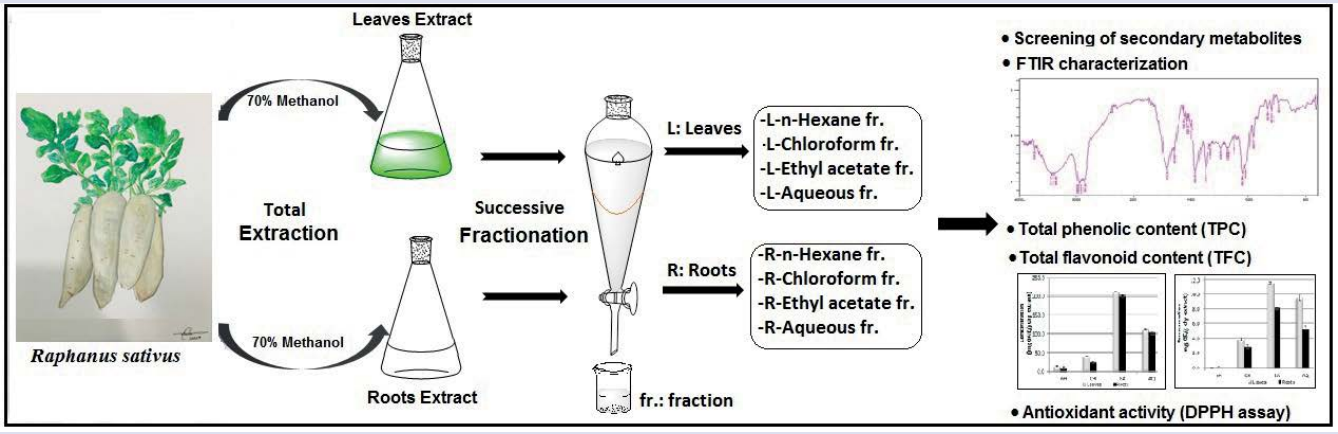
ACKNOWLEDGEMENTS

This work was supported through the Annual Funding track by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Project No. AN000194].

REFERENCES

1. Checa J, Aran JM. Reactive Oxygen Species: Drivers of Physiological and Pathological Processes. J Inflamm Res. 2020;13:1057-1073.
2. Nita M, Grzybowski A. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev. 2016;2016:3164734.
3. Salman KA, Ashraf S. Reactive Oxygen Species: A link between chronic inflammation and cancer. Asia-Pacific J Mol Biol Biotechnol. 2013;21:41-49.
4. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-126.
5. Lourenço SC, Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: From sources to food industry applications. Molecules. 2019;24(22):4132.
6. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5(3):93.
7. Mandaville JP. Flora Of Eastern Saudi Arabia. Routledge, Taylor & Francis Group, Oxford, UK. 1990.
8. Migahid AM. Flora of Saudi Arabia. King Saud University, Riyadh. 1987.
9. Harvey AL. Natural products in drug discovery. Drug Discov Today. 2008;13:894-901.
10. Dhaliwal MS. Handbook of Vegetable Crops. Kalyani Publishers, New Delhi, India. 2007;194-199.
11. 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):1064-1070.
12. 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):127-134.
13. Reddy PV, Shruthi D, Faiyaz A, Asna U. Antioxidant properties and stability of *Raphanus sativus* extracts. J Pharm Res. 2010;3(3):658-661.
14. Park CH, Baskar TB, Park SY, Kim SJ, Valan AM, Al-Dhabi NA, *et al.* Metabolic profiling and antioxidant assay of metabolites from three radish cultivars (*Raphanus sativus*). Molecules. 2016;21(2):157.
15. Raza A, Razzaq A, Mehmood SS, Zou X, Zhang X, Lv Y, *et al.* Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. Plants. 2019;8(2):34.
16. Harborne JB. Phytochemical Methods. Chapman and Hall Ltd, London. 1985;37.
17. Tharu AK, Paudel MR, Joshi AP, Bhandari L, Aryal HP. Screening of Secondary Metabolites and Antioxidant Activity of Wild Edible Termite Mushroom. Pharmacogn J. 2022;14(2):301-307.
18. Khalil HE, Aljeshi YM, Saleh FA, Mohamed TS. Assessment of chemical composition and the antimicrobial and antioxidant activities of *Bassia eriophora* growing in Eastern province of Saudi Arabia. J Chem Pharm Res. 2017;9(2):210-215.
19. Khalil HE, Aljeshi YM, Saleh FA. Authentication of *Carissa macrocarpa* cultivated in Saudi Arabia; botanical, phytochemical and genetic study. J Pharm Sci Res. 2015;7(8):497-508.
20. Khalil HE, Aljeshi YM, Saleh FA. Phytochemical Analysis and *in Vitro* Antioxidant Properties of *Sisymbrium irio* L Growing in Saudi Arabia: A Comparative Study. Res J Pharm Biol Chem Sci. 2017;8(3):2533-2540.
21. Samy MN, Khalil HE, Sugimoto S, Matsunami K, Otsuka H, Kamel MS. Biological studies on chemical constituents of *Ruellia patula* and *Ruellia tuberosa*. J Pharmacogn Phytochem. 2015;4(1):64-67.
22. Oliveira RN, Mancini MC, Oliveira FC, Passos TM, Quilty B, Thiré RM, *et al.* FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. Matéria. 2016;21:767-779.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Hany Ezzat Khalil is a Associate Professor, Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Kingdom of Saudi Arabia. Interested in isolation and identification of bioactive compounds from plants and other natural resources.

Abdullah Abdulhamid Altaweel is a Pharm D. undergraduate student, Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Kingdom of Saudi Arabia.

Abdullah Jalal Alasoom is a Pharm D. undergraduate student, Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Kingdom of Saudi Arabia.

Hussein Ali Burshed is a Pharm D. undergraduate student, Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Kingdom of Saudi Arabia.

Marwan Mohamed Alshawush is a Pharm D. undergraduate student, Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Kingdom of Saudi Arabia.

Cite this article: Altaweel AA,, Alasoom AJ, Burshed HA, Alshawush MM, Khalil HE. Insight 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.