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Research Article

Phytochemical profiling and anti-inflammatory evaluation of *Raphanus raphanistrum* subsp. sativus (L.) extracts

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Abstract

Dysregulated inflammatory responses may result in various chronic diseases. Traditionally, non-steroidal anti-inflammatory drugs (NSAIDs) have been used for the treatment of inflammatory diseases. However, due to their serious adverse effects when used for long-term, the researcher has developed an interest in natural plant-based remedies. The current study investigated the phytochemical composition and anti-inflammatory activity of *Raphanus raphanistrum* subsp. *sativus* (L.), a Brassicaceae member which is traditionally used as a source of nutrition and also against various diseases. The whole plant was collected from Feroza village (District Rahim Yar Khan, Pakistan), shade-dried, powdered, and extracted with two solvents: methanol and dichloromethane (DCM). Preliminary phytochemical screening discovered the presence of alkaloids, cardiac glycosides, terpenoids, and flavonoids, whereas tannins and lipids were absent. Thin-layer chromatography (TLC) and column chromatography analyses yielded multiple fractions rich in bioactive constituents. The DCM extract demonstrated notable *in vitro* anti-inflammatory activity, inhibiting 53.6% of reactive oxygen species (ROS) generation at 100 µg/mL (IC_{50} = 93.5 ± 2.4 µg/mL), compared to ibuprofen (73.2 ± 1.4% inhibition, IC_{50} = 11.2 ± 1.9 µg/mL). *In vivo* experiments were carried out using carrageenan-induced paw edema in Wistar rats. This further confirmed a significant, dose-dependent inhibition of inflammation, which was comparable to that of diclofenac sodium when used at higher doses. These findings validate the ethnomedicinal use of *R. raphanistrum*. Our results demonstrated that this plant can be a promising natural source of anti-inflammatory compounds. However, future studies using advanced spectroscopic and mechanistic analyses are required to identify the specific active constituents and explore their pharmacological mechanisms.

Keywords: *Raphanus Raphanistrum*, Phytochemicals, Anti-Inflammatory, TLC Profiling, Natural Remedies, Plant Secondary Metabolites.

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Introduction

Inflammation is the defense mechanism that protects the body from pathogenic stimuli, including microbial infections, allergens, chemical agents, and physical agents. Inflammation can be acute or chronic, where acute inflammation is a short-term protective response, and chronic inflammation shows a long-term response. Chronic inflammation plays a major role in the development of various diseases [1]. Currently, inflammation is mainly treated with synthetic non-steroidal anti-inflammatory drugs (NSAIDs). Besides the fact that these drugs are effective, their significant adverse effects and high cost accentuate the urgent need for safer, more effective, and accessible alternative treatments [2].

Natural products have been used worldwide for the prevention and treatment of diseases for centuries [3]. The evolution of modern medicine resulted in a vast variety of synthetic drugs that revolutionized healthcare; however, the extensive use of these medicines has revealed considerable drawbacks [4]. Many synthetic drugs are not only expensive but also associated with serious adverse effects such as gastrointestinal irritation, peptic ulcers, hepatotoxicity, nephrotoxicity, and hypersensitivity reactions [5]. Due to these potential side effects, the researchers have developed an interest in bioactive compounds of natural origin, which have equal therapeutic potential with fewer side effects and cost effectiveness [6].

The Brassicaceae family, consisting of 338–360 genera and about 3,700 species, is a diverse group of plants with nutritional and medicinal significance [7]. Members are found worldwide and are commonly used for edible oils, condiments, and traditional medicines [8]. The genus Raphanus, including annual and biennial herbs native to the Mediterranean region, such as *Raphanus raphanistrum*, *R. sativus*, and *R. caudatus* [9], is part of this family. These plants are rich in secondary metabolites like alkaloids (e.g., sinapine), sterols (β-sitosterol and campesterol), and raphanin, which has known antibacterial properties [10]. Besides their nutritional value, species of this genus are also recognized for their pharmacological potential [11, 12].

Raphanus raphanistrum, commonly known as wild radish, is an annual or biennial herb that grows up to 120 cm in height, with swollen, fleshy roots of varying colors, elongated racemes of pink, purple, or white flowers, and fusiform fruits containing ball-shaped seeds [13]. Previous studies have reported that the ethanol extract of this plant is effective against various microbes, including *Staphylococcus aureus*, *Salmonella enteritidis*, *Bacillus cereus*, and *Cronobacter sakazakii* [14]. Note, the anti-inflammatory activity of *R. raphanistrum* has been attributed to the presence of phenolic compounds and fatty acids, particularly linoleic acid.

Considering these activities reported previously, the present study aimed to investigate the anti-inflammatory potential of the *Raphanus raphanistrum*. The objectives of the study were to perform a qualitative phytochemical screening to identify key secondary metabolites such as flavonoids, phenols, and terpenes, and to evaluate their anti-inflammatory activity using both *in vitro* and *in vivo* assays. The results of this study aim to support the traditional use of this plant with scientific evidence and to demonstrate its potential as a source for the development of safe and economical anti-inflammatory drugs.

Materials and Methods

Collection and identification of the plant

The whole plant of *Raphanus raphanistrum* was collected from the Feroza village of Tehsil Khanpur district, Rahim Yar Khan,

and identified by Dr. Zafar Ullah Zafar, Associate Professor of the Institute of Botany, Bahauddin Zakariya University, Multan, Pakistan. After collecting, the pods of the plant were dried in the shade and then ground into fine powder.

Separation and purification of plant material

The extraction process was performed by macerating 400 g of the powdered plant material in appropriate solvents, methanol and dichloromethane. The resulting filtrates were then concentrated under reduced pressure using a rotary evaporator to obtain the crude extract.

For the separation and preliminary purification of the bioactive constituents, thin-layer chromatography (TLC) was performed. Each solvent extract (20 mg) was applied as a sample spot on TLC plates, which were subsequently developed in suitable solvent systems. After elution, the plates were visualized under ultraviolet (UV) light at 254 nm and 366 nm using a UV–VIS illuminator. The retention factor (Rf) values of the resolved spots were calculated for the identification and comparison of the separated phytochemical components.

Thin layer chromatography (TLC)

The separation of phytoconstituents was carried out using thin-layer chromate-graphy (TLC) [15]. A sample solution was prepared by dissolving 20 mg of the methanolic extract in 1 mL of methanol and vortexed at 2500 rpm to obtain a clear solution. Pre-cut silica gel TLC plates (10 × 2 cm) were used, and sample spots (5–10 µL, ≤ 6 mm diameter) were applied using a microcapillary tube about 1 cm from the baseline.

The mobile phase was freshly prepared in the required solvent ratio and placed in a saturated TLC chamber for 15 minutes before development. The spotted plates were then inserted into the chamber, allowing elution by capillary action until the solvent front reached the marked line. Plates were removed, air-dried, and visualized under UV light at 254 nm and 366 nm. The same procedure was repeated for the dichloromethane (DCM) extract, and Rf (retardation factor) values were calculated to characterize the separated components.

Column chromatography

4 g of the dichloromethane (DCM) extract and 8 g of silica gel (2:1) were blended to create a homogenous slurry for column chromatography. A 60 g silica gel slurry in methanol was made separately and meticulously put onto a glass column that had been previously washed with methanol. For uniform packing, the column was let to settle for half an hour.

Ethyl acetate–methanol solutions of different polarities, 80:20 (polar), 82:18 (moderately polar), and 84:16 (least polar), were used for elution. After the mobile phase and stationary phase were clearly separated, the sample mixture was put onto the top of the column, and elution began at a flow rate of 3 milliliters per minute. A total of 35 fractions were collected sequentially.

Thin-layer chromatography (TLC) was used to analyze each fraction in order to calculate the Rf values and evaluate component similarity. Fractions with comparable TLC profiles were combined, condensed with a rotary evaporator, and then placed in sample vials with labels for additional examination.

Preliminary phytochemical screening

Standard methods were used to qualitatively screen for the presence of primary and secondary metabolites in the powdered plant material and its solvent

extracts [16 - 18].

Primary metabolites

Primary metabolites including proteins, lipids, and carbohydrates, were examined using solubility tests for lipids, the Molisch, Fehling, Benedict, and Iodine tests for carbohydrates, and the Ninhydrin, Biuret, and Nitroprusside tests for proteins.

In order to test secondary metabolites, standard phytochemical techniques were used. While cardiac glycosides were found using the Keller–Killiani test, alkaloids were identified utilizing the Dragendorff, Wagner, and Mayer tests. The Salkowski reaction was used to evaluate terpenoids, while the foaming test was used to detect the presence of saponins. While flavonoids were investigated using the alkaline reagent and lead acetate tests, tannins were assessed using ferric chloride, catechin, and gelatin assays.

Based on the distinctive color responses or precipitate formation, the presence or absence of each metabolite was noted.

In vitro anti-inflammatory assay

A luminol-based chemiluminescence assay, which quantifies the production of reactive oxygen species (ROS), was used to assess the plant extracts *in vitro* anti-inflammatory potential. It used luminol as the chemiluminescent probe. Briefly, Hank's balanced salt solution (HBSS) was used to dilute 25 µL of freshly drawn blood, which was then combined with 25 µL of dimethyl sulfoxide (DMSO) and water. After adding 25 µL of luminol and a suitable volume of HBSS, the reaction mixture was stimulated with 25 µL of opsonized zymosan. Assays were performed in 96-well microplates and incubated for 50 minutes. Chemiluminescence was recorded at 30-second intervals using a luminometer, and the results were expressed as relative light units (RLU).

The percentage inhibition of ROS production was calculated using the formula:

% Inhibition = [(RLU control – RLU sample) / RLU control] × 100

In vivo anti-inflammatory activity

Adult Wistar albino rats (60-120 g) of either sex were used for the *in vivo* evaluation of anti-inflammatory activity. Animals were housed under standard laboratory conditions with free access to a balanced diet and water ad libitum. Acute inflammation was induced by sub plantar injection of 0.1 mL carrageenan into the left hind paw. The plant extract was given orally at various concentrations (100, 200, 300, and 400 mg/Kg body weight) 30 minutes before the carrageenan injection. Paw volumes were measured at 60-, 120-, 180-, and 240-minute post-induction using a plethysmograph. The percentage inhibition of paw edema in extract-treated groups was calculated relative to the negative control group. Diclofenac sodium (5 mg/kg, p.o.) served as the reference standard or positive control.

Statistical analysis

All experimental data were analyzed with the GraphPad Prism software (8.0.1). The results are reported as mean ± standard error of the mean (SEM), with sample sizes of 4 for in vitro experiments and 6 for each group in in vivo investigations. Differences across groups were tested using two-way analysis of variance (ANOVA), followed by Duncan's post hoc test to identify significant pairwise comparisons.

Results

Preliminary analysis of Dichloro-methane extract

The preliminary analysis of the phytochemicals in the dichloromethane (DMC) extract of the plant showed the

presence of primary and secondary metabolites. The presence of primary metabolites, including carbohydrates and proteins, was confirmed by performing multiple tests, while lipid was found to be absent in the plant extract (Table 1).

Table 1: Primary metabolites screening from the extract of *R. raphanistrum.*

Primary metabolites	**Inferences**
Carbohydrates	Present
Proteins	Present
Lipids	Very less/Absent

Several secondary metabolites were identified in the extract of *Raphanus raphanistrum* as indicated in (Table 2). These secondary metabolites include alkaloids, glycosides, terpenoids, and flavonoids. Cardiac glycosides were found more compared to saponin glycosides, which were present in very small quantities. More than one test was performed for the confirmation of these metabolites.

Table 2**:** Secondary metabolites screening from the extract of *R. raphanistrum.*

Secondary metabolites	**Inferences**
Alkaloids	Alkaloid present
Cardiac glycosides	Cardiac glycosides present
Saponin glycosides	Very small saponin
Terpenoids	Terpenoids are present
Flavonoids	Flavonoids are present
Tannins	Tannins are absent

Thin-layer chromatography analysis

Thin-layer chromatography analysis of the Dichloromethane extract of the Raphanus raphanistrum plant resulted in various fractions. TLC analysis was conducted on the dichloromethane and methanol extracts of Raphanus raphanistrum to identify active constituents. Aluminum-backed silica plates were used. In the initial stage, several binary mobile phases were tested. The most effective resolution for the dichloromethane extract came with ethyl acetate: methanol (80:20), separating seven spots with Rf values 0.12, 0.21, 0.42, 0.54, 0.68, 0.77, and 0.82 at 366 nm, and one spot at 254 nm. A subsequent tertiary system, chloroform: methanol: water (85:10:1), gave better separation: four components with Rf 0.29, 0.56, 0.74, 0.94 at 366 nm, and five components with Rf 0.29, 0.46, 0.56, 0.74, 0.88 at 254 nm. Upon spraying with Godin's reagent followed by 10% sulfuric acid, three components appeared at Rf 0.46, 0.74, and 0.94 (Table 3).

The methanol extract showed optimal separation with binary mobile phases using toluene: ethyl acetate (80:20), yielding four components with Rf values 0.24, 0.42, 0.64, and 0.81 at 366 nm, and two components with Rf 0.64 and 0.81 at 254 nm. With a tertiary system of ethyl acetate: methanol: water (70:30:4), seven components were resolved with Rf values 0.10, 0.14, 0.29, 0.41, 0.56, 0.73, and 0.90 at 366 nm, and four components with Rf values 0.14, 0.56, 0.64, 0.73, and 0.90 at 254 nm. After Godin's reagent treatment, three components appeared with Rf values 0.56, 0.64, and 0.73 (Table 3).

Column chromatography resulted in 9 subfractions of the extract

The 2g open column chromatography was used to analyze the dichloromethane extract of the plant. The stationary phase used was silica gel (60,70-230 mesh).

Table 3: TLC Analysis of dichloromethane and methanol extracts under different mobile phase systems.

Extract Type	Mobile Phase System	Mobile Phase Ratio	Wave-length (nm)	No. of Components	Rf Values	Visualization / Reagent
Dichloro-methane Extract	Ethyl acetate: Methanol	80:20:00	366	7	0.12, 0.21, 0.42, 0.54, 0.68, 0.77, 0.82	UV light
			254	1	—	UV light
	Chloroform : Methanol : Water	85:10:01	366	4	0.29, 0.56, 0.74, 0.94	UV light
			254	5	0.29, 0.46, 0.56, 0.74, 0.88	UV light
			—	3	0.46, 0.74, 0.94	After spraying with Godin's reagent and 10% H_2 SO_4
Methanol Extract	Toluene : Ethyl acetate	80:20:00	366	4	0.24, 0.42, 0.64, 0.81	UV light
			254	2	0.64, 0.81	UV light
	Ethyl acetate : Methanol : Water	70:30:04	366	7	0.10, 0.14, 0.29, 0.41, 0.56, 0.73, 0.90	UV light
			254	5	0.14, 0.56, 0.64, 0.73, 0.90	UV light
			—	3	0.56, 0.64, 0.73	After spraying with Godin's reagent

Mobile phase used with a ratio of ethyl acetate(80): methanol(20). 35 fractions were obtained with each fraction of 15ml volume. These 35 fractions were examined by TLC. Depending on the TLC analysis, similar fractions were mixed together, and 9 subfractions were obtained (Table 4).

Table 4: The weights of 9 subfractions are given below. (F: Fraction, RRPD: *Raphanus raphanistrum* pods dichloromethane).

Fractions combined	Name of sub fraction	Weight (gm)
F2,F3,F4,F5	RRPD-A	0.2
F6,F7,F8	RRPD-B	0.7
F9,F10,F11,F12, F13,F14	RRPD-C	0.8
F24,F29	RRPD-D	0.1
F30,F32	RRPD-E	0.13
F15,F16,F17, F18,F19	RRPD-F	0.4
F20,F23	RRPD-G	0.11
F33,F35	RRPD-H	0.15
F21,25	RRPD-I	0.11

DCM extract of *Raphanus raphanistrum* showed Anti-inflammatory properties

Anti-inflammatory activity for the *R. raphanistrum* plant by using 1mg of the Dichloromethane extract of the drug was evaluated. Dichloromethane extract showed 53.6% anti-inflammatory activity at a concentration of 100 µg/ml. Ibuprofen was used as standard treatment, which showed 73.2% inhibition at a concentration of 25 µg/ml (Table 5).

Anti-inflammatory activity was also measured in rats, where paw inflammation was induced by carrageenan. The DCM extract of *R. raphanistrum* exhibited a promising anti-inflammatory activity starting at a dose of 200 mg/kg body weight. The significant reduction in the paw oedema occurred after 60 minutes, with a persistent effect remaining until four hours. This activity was comparable to the standard drug diclofenac sodium (Table 6).

Table 5: IC50 values of DCM extract of the drug for anti-inflammatory activity in vitro.

Variables	**Standard drug (Ibuprofen)**	**Dichloromethane extract of the drug (RRPD)**
Concentration (µg/ml)	25 µg/ml	100 µg/ml
% inhibition/ stimulation	73.2 ± 1.4 %	53.60%
IC50 ± SD	11.2 ± 1.9 µg/ml	93.5 ± 2.4 µg/ml

IC: inhibition concentration, **SD:** standard deviation, **RRPD**: *Raphanus raphanistrum* pods dichloromethane (n=4).

Table 6: Anti-inflammatory activity of DCM extract of *R. raphanistrum* against carrageenan-induced oedema at different concentrations and time points.

		Carrageenan-induced oedema (volume in ml)			
		Mean ± SEM %			
Drug	**Dose mg/kg**	**60min**	**120min**	**180min**	**240min**
Control	0	0.38±0.01	0.39±0.05	0.45±0.01	0.46±0.009
Diclofenac	5	0.19±0.03***	0.18±0.01***	0.17±0.04****	0.13±0.07****
RRPD	100	0.31±0.07*	0.33±0.03*	0.35±0.02*	0.36±0.03*
RRPD	200	0.22±0.05**	0.23±0.04**	0.21±0.03***	0.24±0.04**
RRPD	300	0.23±0.010**	0.24±0.02**	0.23±0.03**	0.20±0.001***
RRPD	400	0.18±0.03***	0.17±0.04****	0.20±0.07***	0.21±0.02***

The data are shown as Mean±SE, where n = 6 for each group. Comparison is made between the control group and other treatments where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Discussion

The present study aimed to explore the phytochemical composition and pharmacological potential of *Raphanus raphanistrum*, an underexplored member of the Brassicaceae family known for its bioactive secondary metabolites. Although several studies have reported the biological properties of this species [13, 14], many of its phytoconstituents, their pharmacological activities, and mechanisms of action remain insufficiently characterized. Therefore, this research is expected to contribute to the existing knowledge by identifying its major phytochemical constituents and relating them to its pharmacological relevance.

Preliminary phytochemical screening revealed the presence of carbohydrates, reducing sugars, glycine, and cysteine, while lipids were absent. The plant contained secondary metabolites such as alkaloids, cardiac glycosides, and flavonoids, but no saponins or tannins. These findings indicate that R. raphanistrum could be a useful natural source for the extraction and purification of potentially therapeutically relevant bioactive secondary metabolites in future studies.

Column chromatography of the dichloromethane extract was performed using thin-layer chromatography (TLC) profiling, yielding 35 fractions. These fractions were subsequently divided into eight subfractions based on chromatographic similarities. These subfractions can serve as a foundation for additional structural investigation and biological testing of specific chemicals [19].

The presence of flavonoids and phenolic substances was validated using various phytochemical techniques. These chemicals are especially noteworthy due to their well-documented anti-inflammatory and antioxidant effects [20 - 23]. Previous studies have shown that *R. sativum*, which is a close relative of *R. raphanistrum*, extracts reduce the expression of cyclooxygenase-2 (COX-2) and nuclear factor-kappa B (NF-κB), thereby exerting potent anti-inflammatory effects [24]. Another study on *R. sativum* demonstrated that it exerted anti-inflammatory activity by negatively regulating the p38 and mitogen-activated protein kinase (MAPK) pathways, specifically the MAPK-NF-κB pathway [25]. The observed phytochemical profile of our plant resembles that of R. sativum, suggesting a comparable pharmacological potential. The confirmation of the presence of isothiocyanates, flavonoids, and alkaloids in the current study may contribute to the diverse biological actions of *R. raphanistrum*, reflecting the multipurpose therapeutic nature of this plant. The anti-bacterial activity of the plant was also reported previously, where the extracts of this plant were active against both gram-negative and gram-positive bacteria and found non-toxic to the non-tumor cells. This anti-bacterial activity would be due to the presence of fourteen phenolic compounds, in which kaempferol-3,7-*O*-di-rhamnoside was found to be the most abundant [13, 26].

Taken together, our results also highlight *R. raphanistrum* as a biologically rich source of phytoconstituents with potential pharmacological applications. The observed anti-inflammatory activity was validated through both *in vitro* (the Luminol-based chemiluminescence assay) and *in vivo* experiments. The results supported the potential of this plant as a candidate for drug development or nutraceutical formulation for managing inflammatory disorders such as rheumatoid arthritis.

Despite these promising findings, the present study remains preliminary in scope. While qualitative phytochemical screening and TLC profiling provided valuable

insights, quantitative estimations and advanced spectroscopic analyses (e.g., GC-MS, LC-MS, NMR) are necessary to precisely characterize the bioactive compounds. Furthermore, the mechanistic pathways underlying the observed anti-inflammatory effects require detailed investigation through in vitro and in vivo models, including enzyme inhibition assays and cytokine profiling.

Future studies should also focus on the toxicological assessment and pharmacokinetic behavior of *R. raphanistrum* extracts to ensure their safety and efficacy. Establishing a clear correlation between specific compounds and biological activities will be crucial to translating these findings into therapeutic applications.

Conclusion

The study provides preliminary but compelling evidence that the dichloromethane extract of wild radish (Raphanus raphanistrum) exhibits anti-inflammatory activity, as shown by in vitro reactive oxygen species inhibition (53.6% at 100 µg/mL; IC50 ≈ 93.5 µg/mL) and dose-dependent reduction of carrageenan-induced paw edema in rats, with effects approaching those of diclofenac at higher doses. Phytochemical analyses identify several bioactive classes (alkaloids, cardiac glycosides, flavonoids, terpenoids), suggesting the presence of active constituents in multiple fractions.

References

1. Chavda VP, Feehan J, Apostolopoulos V. Inflammation: The cause of all diseases. Cells. 2024 Nov 18;13(22):1906.
2. Wirth T, Lafforgue P, Pham T. NSAID: Current limits to prescription. Joint Bone Spine. 2024 Jul 1;91(4):105685.
3. Chaachouay N, Zidane L. Plant-derived natural products: a source for drug discovery and development. Drugs and Drug Candidates. 2024 Feb 19;3(1):184-207.
4. Karimi A, Majlesi M, Rafieian-Kopaei M. Herbal versus synthetic drugs; beliefs and facts. Journal of nephro-pharmacology. 2015 Jan 1;4(1):27.
5. Saggar S, Mir PA, Kumar N, Chawla A, Uppal J, Kaur A. Traditional and herbal medicines: opportunities and challenges. Pharmacognosy Research. 2022;14(2).
6. Leitzmann C. Characteristics and health benefits of phytochemicals. Forschende Komplementärmedizin/Research in Complementary Medicine. 2016 Feb;23(2):69-74.
7. Raza A, Hafeez MB, Zahra N, Shaukat K, Umbreen S, Tabassum J, Charagh S, Khan RS, Hasanuzzaman M. The plant family Brassicaceae: Introduction, biology, and importance. The Plant Family Brassicaceae: Biology and Physiological Responses to Environmental Stresses. Springer. 2020: 1-43.
8. Ross IA. Plant-Based Therapeutics, Volume 2: The Brassicaceae Family. Springer Nature; 2024 Oct 18.
9. Kaneko Y, Bang SW, Matsuzawa Y. Raphanus. In: Wild Crop Relatives: Genomic and Breeding Resources – Vegetables. Springer; 2011: 247-258.
10. Park CH, Baskar TB, Park SY, Kim SJ, Valan Arasu M, Al-Dhabi NA, Kim JK, Park SU. Metabolic profiling and antioxidant assay of metabolites from three radish cultivars (Raphanus sativus). Molecules. 2016 Jan 28;21(2):157.
11. Gamba M, Asllanaj E, Raguindin PF, Glisic M, Franco OH, Minder B, Bussler W, Metzger B, Kern H, Muka T. Nutritional and phytochemical characterization of radish (Raphanus sativus): A systematic review. Trends in Food Science & Technology. 2021 Jul 1; 113:205-218.
12. Hanlon PR, Barnes DM. Phytochemical composition and biological activity of 8

varieties of radish (Raphanus sativus L.) sprouts and mature taproots. Journal of Food Science. 2011;76(1):C185-C192.
13. Iyda JH, Fernandes A, Ferreira FD, Alves MJ, Pires TC, Barros L, Amaral JS, Ferreira IC. Chemical composition and bioactive properties of the wild edible plant Raphanus raphanistrum L. Food Research International. 2019 Jul 1; 121:714-722.
14. Lim HW, Song KY, Chon JW, Jeong D, Seo KH. Antimicrobial action of Raphanus raphanistrum subsp. sativus (radish) extracts against foodborne bacteria present in various milk products: A Preliminary Study. Journal of Dairy Science and Biotechnology. 2019;37(3):187-195.
15. Kagan IA, Flythe MD. Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. Journal of visualized experiments: JoVE. 2014 Mar 27(85):51411.
16. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998 Apr 30.
17. Gokhale MS, Kokate C, Bidarkar D. Pharmaceutical Biology. Pragati Books Pvt. Ltd. 2008.
18. Evans WC. Trease and Evans' pharmacognosy. Elsevier Health Sciences; 2009 May 27.
19. Kowalska T, Sajewicz M. Thin-layer chromatography (TLC) in the screening of botanicals–its versatile potential and selected applications. Molecules. 2022 Oct 5;27(19):6607.
20. 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 Aug 25;5(3):93.
21. Xu YB, Chen GL, Guo MQ. Antioxidant and anti-inflammatory activities of the crude extracts of Moringa oleifera from Kenya and their correlations with flavonoids. Antioxidants. 2019 Aug 9;8(8):296.
22. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as potential anti-inflammatory molecules: A review. Molecules. 2022 May 2;27(9):2901.
23. Chagas MD, Behrens MD, Moragas-Tellis CJ, Penedo GX, Silva AR, Gonçalves-de-Albuquerque CF. Flavonols and flavones as potential anti-inflammatory, antioxidant, and antibacterial compounds. Oxidative medicine and cellular longevity. 2022;2022(1):9966750.
24. Park HJ, Song M. Leaves of Raphanus sativus L. shows anti-inflammatory activity in LPS-stimulated macrophages via suppression of COX-2 and iNOS expression. Preventive nutrition and food science. 2017 Mar 31;22(1):50.
25. Kook SH, Choi KC, Lee YH, Cho HK, Lee JC. Raphanus sativus L. seeds prevent LPS-stimulated inflammatory response through negative regulation of the p38 MAPK-NF-κB pathway. International immunopharmacology. 2014 Dec 1;23(2):726-734.
26. Mohamed Z, Nawrooz M, Hlail H, Hamad M, Hamadi S, Jaber S. An Extraction, Characterization and Evaluation of Antimicrobial Effectiveness of Raphanus Sativus Extracts. The Continents States University Journal. 2024 Mar 31;1(1.0):14-18.