Journal of Health and Rehabilitation Research 2791-156X JHRR

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Original Article

Antioxidant, Immunomodulatory and Fungicidal Potential of Different Extracts of Raphanus Raphanistrum L. Var. Caudatus

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Conflict of Interest: None.

*Akhlaq M., et al. (2024). 4(1): **DOI**: https://doi.org/10.61919/jhrr.v4i1.441*

ABSTRACT

Background: Reactive oxygen species (ROS) have been implicated in the pathogenesis of various diseases, emphasizing the importance of antioxidants in maintaining cellular homeostasis. Raphanus raphanistrum, commonly known as wild radish, has been traditionally used for its medicinal properties, including antioxidant, immunomodulatory, and antifungal effects. The plant is rich in bioactive compounds such as flavonoids, isothiocyanates, and vitamins, which contribute to its therapeutic potential.

Objective: This study aimed to evaluate the antioxidant, immunomodulatory, and antifungal activities of Raphanus raphanistrum extracts and to compare these activities with standard controls.

Methods: Fresh Raphanus raphanistrum plants were collected, and extracts were prepared using methanol, chloroform, and water. The antioxidant activity was assessed using the DPPH radical scavenging assay. Immunomodulatory activity was evaluated through the luminol-enhanced chemiluminescence assay in whole blood and polymorphonuclear leukocytes (PMNS), measuring the oxidative burst. The antifungal activity was determined using the agar disk diffusion method against six pathogenic fungi. The statistical analysis was performed using SPSS version 25, with results presented as mean ± standard deviation for antioxidant and antifungal assays, and mean ± standard error for immunomodulatory activity.

Results: The methanol extract exhibited significant antioxidant activity with DPPH scavenging percentages ranging from 56.55% at 150 µg/ml to 87.08% at 850 µg/ml. Immunomodulatory activity showed IC50 values of 37.8 µg/ml in whole blood and 18.9 µg/ml in PMNS for the methanol extract. The antifungal activity against Candida albicans showed zones of inhibition increasing from 15 mm at 150 µg/ml to 22 mm at 850 µg/ml. These activities were compared to standard controls, including ascorbic acid for antioxidant, ibuprofen for immunomodulatory, and miconazole for antifungal assays, demonstrating the extracts' competitive potential.

Conclusion: Raphanus raphanistrum extracts showed promising antioxidant, immunomodulatory, and antifungal activities, indicating their potential as natural alternatives for the prevention and treatment of related diseases. Further in vivo studies and isolation of active compounds are recommended to fully understand the mechanisms and therapeutic applications.

Keywords: Raphanus Raphanistrum, Antioxidant Activity, Immunomodulatory Activity, Antifungal Activity, Agar Disk Diffusion, Uman Healthcare.

INTRODUCTION

Oxygen's interaction with specific molecules can lead to the generation of free radicals, which then stimulate the production of reactive oxygen species (ROS). These ROS are highly reactive and capable of initiating chain reactions that may damage essential cellular components such as DNA, potentially leading to cell death. Such unchecked ROS levels are linked to a myriad of illnesses, including aging, inflammation, cancer, infertility, arthritis, stroke, atherosclerosis, and various neurological disorders (1). Antioxidants

Journal of Health and Rehabilitation Research 2791-156X JHRR

serve as a crucial defense mechanism against the proliferation of ROS in the body, with certain enzyme systems and vital vitamins like vitamin E and C playing significant roles in scavenging free radicals. In light of the current health challenges associated with aging and free radical damage, there is a growing interest in developing antioxidants from natural compounds (2).

Medicinal plants, including Raphanus raphanistrum, have been identified for their potent antioxidant capabilities. Known commonly as wild radish, R. raphanistrum has been utilized since ancient times for its nutritional and medicinal properties. It is also referred to as white charlock and is a member of the Brassicaceae family. The plant, including its aerial parts like pods, has been extensively studied for its antioxidant potential across various varieties (3). The root of R. raphanistrum, in particular, has been used to address cardiovascular diseases, diabetes, cancer, and gastrointestinal issues, showcasing hepatoprotective properties. Research has highlighted the presence of antioxidant properties in its leaves, roots, and seeds. Additionally, radish is known for its rich content of isothiocyanates, flavonoids, and phenolic acids, demonstrating significant immunomodulatory and anti-inflammatory effects both in vitro and in vivo (4, 5).

The activation of phagocytes, a crucial immune response, leads to increased cellular consumption of molecular oxygen. This process, mediated by the enzyme system NADPH-oxidase, results in the production of ROS. While these ROS are beneficial for host defense, they can also have toxic effects on cells. Thus, proper immunomodulation is essential for the prevention of various diseases (6). To assess the radical scavenging and immunomodulatory activities of R. raphanistrum, this study employs DPPH, luminol-enhanced chemiluminescence assays, and the agar disk diffusion test. These methods are instrumental in evaluating the plant's capacity to neutralize free radicals, modulate immune responses, and exhibit fungicidal activity, respectively. This research aims to provide a comprehensive understanding of the potential health benefits associated with R. raphanistrum, emphasizing its role in managing diseases related to oxidative stress and immune system dysregulation.

MATERIAL AND METHODS

Fresh and healthy specimens of Raphanus raphanistrum were collected from Karachi, District Sindh, Pakistan. The plant material underwent a meticulous cleaning process using double distilled water to ensure purity before being air dried, sliced, and stored at a controlled temperature of 25°C in airtight containers to prevent degradation and contamination, all while being properly labeled to facilitate identification and subsequent analyses (8).

The extraction process involved grinding the dried plant material using a mechanical grinder to facilitate the extraction of bioactive compounds. Approximately 1 kg of the herb was subjected to extraction using solvents such as methanol, chloroform, and water through the method of maceration over a period of 7 days. Following the maceration, the mixtures were filtered using autoclaved Whatman's filter paper and centrifuged at 2400 × g for 15 minutes to separate the supernatant from the particulate matter. The filtered extracts were then evaporated under reduced pressure using a rotary evaporator (Buchi) at 45°C to obtain dry extracts. The yield of each extract was calculated as a percentage of the starting material and the extracts were stored at 4°C until further analysis to maintain their integrity and bioactivity (8).

The antioxidant activity of R. raphanistrum extracts was evaluated using the DPPH radical scavenging assay, a widely recognized method for assessing the free radical scavenging capacity of various substances. Different concentrations of the extracts (150, 250, 450, and 850 µg/ml) were prepared in DMSO and reacted with a DPPH solution prepared in methanol. The mixture was incubated at 37°C for 30 minutes and the reduction in DPPH absorbance was measured at 517 nm using a multiplate reader (SpectraMax340), indicating the antioxidant capacity of the extracts. Vitamin C was employed as a positive control to benchmark the antioxidant activity of the extracts (9).

The immunomodulatory potential of the extracts was determined through a chemiluminescence assay enhanced by luminol, designed to quantify the oxidative burst of phagocytes, a critical aspect of the immune response. This assay involved incubating diluted whole blood and PMNs with varying concentrations of the extracts and assessing the production of reactive oxygen species using luminol as a probe. The chemiluminescence produced was measured, providing insight into the immunomodulatory activity of the extracts (7).

Antifungal activity was assessed using the agar disk diffusion method against six pathogenic fungi. The extracts were sterilized, dissolved in DMSO, and applied to inoculated agar plates. The zones of inhibition were measured after incubation, providing a quantitative measure of the fungicidal activity of the extracts. Miconazole served as a reference antibiotic for comparison, and methanol was tested separately to ensure it did not influence the results. The experiments were conducted in triplicate to ensure reliability of the data (10).

Data collected from these assays were analyzed using the Statistical Package for the Social Sciences (SPSS) version 25. The antioxidant activity results were expressed as mean ± standard deviation (SD), while the immunomodulatory activity data were presented as

Journal of Health and Rehabilitation Research 2791-156X JHRR

median inhibitory concentrations (IC) with standard error. The significance of differences between groups was determined using ANOVA, with a p-value of less than 0.05 considered statistically significant.

In addition to the experimental procedures, the study adhered to ethical guidelines in line with the Declaration of Helsinki for research involving plant materials, ensuring that collection and research methodologies were conducted responsibly and ethically. This comprehensive approach to the study's material and methods ensures the reliability and validity of the findings regarding the antioxidant, immunomodulatory, and antifungal potential of R. raphanistrum extracts, providing a solid foundation for further research in these areas.

RESULTS

The study investigated the free radical scavenging activity of Raphanus raphanistrum extracts and compared it with the well-known antioxidant, ascorbic acid, using the DPPH assay. The methanol extract showed a significant increase in antioxidant activity with increasing concentration, starting from 56.55% at 150 µg/ml to 87.08% at 850 µg/ml (Table 1). This trend was observed across all extracts, with the chloroform extract displaying a scavenging activity that ranged from 19.76% at the lowest concentration to 76.30% at the highest concentration. The aqueous extract exhibited activity only at higher concentrations, with 34.09% at 450 µg/ml and 54.21% at 850 µg/ml, indicating a concentration-dependent increase in free radical scavenging capability. Ascorbic acid, used as a positive control, consistently showed high antioxidant activity, ranging from 95.81% to 97.60%, underscoring the benchmark of effective antioxidant capacity.

In assessing the immunomodulatory activity of R. raphanistrum extracts, the methanol extract demonstrated notable potency with an IC50 of 37.8 µg/ml in whole blood and 18.9 µg/ml in polymorphonuclear leukocytes (PMNS), suggesting a strong effect in modulating the oxidative burst (Table 2). The chloroform and aqueous extracts exhibited less potency, with IC50 values significantly higher, particularly in the aqueous extract, which showed the least immunomodulatory activity with IC50 values of 284.4 µg/ml in whole blood and 142.2 µg/ml in PMNS. Ibuprofen, serving as a positive control, presented with the lowest IC50 values, 11.3 µg/ml in whole blood and 2.88 µg/ml in PMNS, highlighting its well-documented anti-inflammatory effects.

Table 1 Free Radical Scavenging Activity of Raphanus raphanistrum Extracts and Ascorbic Acid by DPPH Assay

Concentrations (µg/ml)	Methanol Extract (%)	Chloroform Extract (%)	Aqueous Extract (%)	Vitamin C (Positive Control) (%)
150	56.55 ± 0.2	19.76 ± 1.7	0.0 ± 0.0	95.81 ± 0.0
250	76.22 ± 0.7	38.84 ± 1.1	0.0 ± 0.0	96.65 ± 0.3
450	85.16 ± 0.7	55.32 ± 1.8	34.09 ± 2.4	97.60 ± 0.5
850	87.08 ± 2.2	76.30 ± 1.2	54.21 ± 3.8	96.79 ± 0.5
*Values are mean ± SD of three experiments.				

Table 2 Immunomodulatory Activity of Raphanus raphanistrum and Ibuprofen using Luminol-enhanced Chemiluminescence Assay

S. No	Extract	Oxidative Burst IC50 (µg/ml) Whole Blood	Oxidative Burst IC50 (µg/ml) PMNS
1	Methanol	37.8 ± 2.1	18.9 ± 2.8
2	Chloroform	136.2 ± 3.1	68.1 ± 2.3
3	Aqueous	284.4 ± 44.8	142.2 ± 0.7
4	Ibuprofen (Control)	11.3 ± 1.89	2.88 ± 0.8
*Values are mean ± SEM of three experiments.			

Table 3 Fungicidal Activity of Miconazole (Standard Drug) Against Different Fungal Strains

S. No	Name of Fungus	150 µg/ml	250 µg/ml	450 µg/ml	850 µg/ml
1	Aspergillus niger	16	20	21	25
2	Trichphyton rubrum	18	19	22	24
3	Microsporum canis	16	17	20	21
4	Fusarium lini	17	18	19	24
5	Candida glabrata	17	19	22	25

Journal of Health and Rehabilitation Research JHRR 2791-156X

S. No	Name of Fungus	150 µg/ml	250 µg/ml	450 µg/ml	850 µg/ml
6	Candida albicans	16	20	24	27
*Values are mean ± SD of three experiments.					

Table 4 Fungicidal Activity of R. raphanistrum Methanolic Extract Against Different Fungal Strains

S. No	Name of Fungus	150 µg/ml	250 µg/ml	450 µg/ml	850 µg/ml
1	Aspergillus niger	13	14	16	19
2	Trichphyton rubrum	13	14	15	17
3	Microsporum canis	12	12	13	14
4	Fusarium lini	-	-	13	15
5	Candida glabrata	14	15	16	18
6	Candida albicans	15	17	19	22
*Values are mean ± SD of three experiments; "-" indicates no zone of inhibition					

The fungicidal activity of R. raphanistrum methanolic extract was further explored against a variety of fungal strains, revealing a spectrum of inhibitory effects. Notably, the extract displayed moderate activity against Aspergillus niger, with zones of inhibition increasing from 13 mm at 150 µg/ml to 19 mm at 850 µg/ml (Table 4). Similar trends were observed against other fungal pathogens, including Trichphyton rubrum and Candida albicans, with the latter showing zones of inhibition expanding from 15 mm at 150 µg/ml to 22 mm at 850 µg/ml. It is interesting to note that the standard drug, miconazole, exhibited stronger fungicidal activity across all tested strains, with inhibition zones for Candida albicans reaching up to 27 mm at the highest concentration (Table 3), thereby setting a comparative standard for antifungal efficacy.

These findings collectively underscore the potential of R. raphanistrum extracts, particularly the methanolic extract, in providing a natural source of antioxidant, immunomodulatory, and fungicidal agents. The concentration-dependent responses observed across the assays highlight the importance of optimizing extract concentrations for maximal biological activity, setting a foundation for future research into the therapeutic applications of R. raphanistrum.

DISCUSSION

In this investigation, the ethanol extract of Raphanus raphanistrum demonstrated significant free radical scavenging and immunomodulatory activities, potentially due to the presence of a rich array of phytochemicals such as lignans, flavonoids, and isothiocyanates (15-17). These compounds are known for their ability to donate protons in the DPPH assay, a property that underpins their antioxidant capabilities. The radish has been traditionally recognized for its therapeutic benefits, including hypolipidemic, anticancer, and antihypertensive effects, attributable to its diverse bioactive compounds (16). Furthermore, the presence of essential nutrients and vitamins in R. raphanistrum enhances its role as a natural antioxidant source, which has been corroborated by studies estimating its total phenolic content and demonstrating its effectiveness in scavenging free radicals (18).

Comparative analyses with other studies reveal a variance in antioxidant potential among different extracts of R. raphanistrum, suggesting that the extraction method, the solvent used, and environmental factors can significantly influence the phytochemical profile and, consequently, the biological activity of the extracts (20-21). For instance, the use of methanol in the current study yielded extracts with notable antioxidant outcomes, contrasting with studies where different solvents might not have efficiently extracted certain phenolic compounds. This discrepancy underscores the critical role of the extraction process in maximizing the therapeutic potential of plant materials.

The immunomodulatory capacity of R. raphanistrum, as demonstrated through the modulation of ROS in vitro, aligns with findings from other studies where radish extracts showed protective effects against immunotoxicity induced by various agents (5, 25). The presence of flavonoids and isothiocyanates in the radish root is believed to contribute to this immunomodulatory activity, emphasizing the importance of these phytochemicals in enhancing the body's defense mechanisms against pathogens and reducing inflammation.

The study also highlighted the antifungal properties of R. raphanistrum, which have been previously documented in various radish varieties. The efficacy of radish extracts against fungal pathogens like Candida albicans can be attributed to isothiocyanates and peptides with fungicidal properties (28-30). These findings provide a foundation for the development of natural antifungal agents, particularly important for populations susceptible to fungal infections, such as individuals with compromised immune systems.

While this investigation establishes a solid basis for considering R. raphanistrum as a potential source of natural antioxidants, immunomodulators, and antifungal agents, it also acknowledges the necessity for further in vivo studies and the isolation of active

Journal of Health and Rehabilitation Research 2791-156X JHRR

compounds to fully elucidate its mechanisms of action. The variability in the biological activity of extracts due to different extraction methods and environmental factors presents both a limitation and an opportunity for optimizing extraction techniques to harness the plant's full therapeutic potential. Additionally, the study's reliance on in vitro assays highlights the need for comprehensive in vivo evaluations to confirm the safety and efficacy of R. raphanistrum extracts in clinical settings.

CONCLUSION

In conclusion, R. raphanistrum holds promise as a lead candidate for developing natural therapeutic agents. However, advancing from in vitro findings to practical applications will require overcoming the challenges associated with standardizing extract preparations and validating the therapeutic benefits through rigorous clinical trials. Future research should aim to identify and isolate the active compounds responsible for the observed biological activities, facilitating the development of standardized and effective formulations for the prevention and treatment of diseases.

REFERENCES

1. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118.
2. Najmi AK, Pillai KK, Pal SN, Aqil M. Free radical scavenging and hepatoprotective activity of jigrine against galactosamine induced hepatopathy in rats. J Ethnopharmacol. 2005;97(3):521–5.
3. Khare CP. Indian medicinal plants: an illustrated dictionary. Springer Science & Business Media; 2008.
4. Reddy PV, Desai S, Ahmed F, Urooj A. Antioxidant properties and stability of Raphanus sativus extracts. J Pharm Res. 2011;3(3):658–61.
5. Salah-Abbès J Ben, Abbès S, Ouanes Z, Houas Z, Abdel-Wahhab MA, Bacha H, et al. Tunisian radish extract (Raphanus sativus) enhances the antioxidant status and protects against oxidative stress induced by zearalenone in Balb/c mice. J Appl Toxicol An Int J. 2008;28(1):6–14.
6. Khan S, Halim SA, Kashif M, Jabeen A, Asif M, Mesaik MA, et al. In-vitro immunomodulatory and anti-cancerous activities of biotransformed products of Dianabol through Azadirachta indica and its molecular docking studies. Chem Cent J. 2013;7(1):1–11.
7. Dahlgren C, Karlsson A. Respiratory burst in human neutrophils. J Immunol Methods. 1999;232(1–2):3–14.
8. Wittschier N, Faller G, Hensel A. Aqueous extracts and polysaccharides from liquorice roots (Glycyrrhiza glabra L.) inhibit adhesion of Helicobacter pylori to human gastric mucosa. J Ethnopharmacol. 2009;125(2):218–23.
9. Kim J-S, Lee Y-S. Antioxidant activity of Maillard reaction products derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. Food Chem. 2009;116(1):227–32.
10. Rios J-L, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: a review of the literature. J Ethnopharmacol. 1988;23(2–3):127–49.
11. Hou W-C, Lin R-D, Cheng K-T, Hung Y-T, Cho C-H, Chen C-H, et al. Free radical-scavenging activity of Taiwanese native plants. Phytomedicine. 2003;10(2–3):170–5.
12. Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. J Agric Food Chem. 2000;48(8):3597–604.
13. Karadag A, Ozcelik B, Saner S. Review of methods to determine antioxidant capacities. Food Anal Methods. 2009;2(1):41–60.
14. Roginsky V, Lissi EA. Review of methods to determine chain-breaking antioxidant activity in food. Food Chem. 2005;92(2):235–54.
15. Muthuswamy U, Ajith MP, Kuppusamy A, Thirumalaiswamy S, Varadharajan S, Puliyath J, et al. In vitro angiotensin converting enzyme inhibitory and antioxidant activities of seed extract of Apium graveolens Linn. Ann Biol Res. 2012;3(3):1274–82.
16. Kunnumakkara AB. Anticancer properties of fruits and vegetables: A scientific review. 2014;
17. Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish J Biol. 2006;30(3):177–83.
18. TURAN NN, AYDIN A. Antioxidant activity and total phenolic content of aqueous extract from Raphanus Raphanistrum L. Turk J Pharm Sci. 2012;9(1):93–100.
19. Chaturvedi P. Inhibitory response of Raphanus sativus on lipid peroxidation in albino rats. Evidence-Based Complement Altern Med. 2008;5(1):55–9.
20. Charoonratana T, Settharaksa S, Madaka F, Songsak T. Screening of antioxidant activity and total phenolic content in Raphanus sativus pod. Int J Pharm Pharm Sci. 2014;6:224–6.

Journal of Health and Rehabilitation Research 2791-156X JHRR

21. Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. J Agric Food Chem. 1996;44(3):701–5.
22. Baek S-H, Park M, Suh J-H, Choi H-S. Protective effects of an extract of young radish (Raphanus sativus L) cultivated with sulfur (sulfur-radish extract) and of sulforaphane on carbon tetrachloride-induced hepatotoxicity. Biosci Biotechnol Biochem. 2008;72(5):1176–82.
23. Pocasap P, Weerapreeyakul N, Barusrux S. Cancer preventive effect of Thai rat-tailed radish (Raphanus sativus L. var. caudatus Alef). J Funct Foods. 2013;5(3):1372–81.
24. Gea-Banacloche JC. Immunomodulation. In: Principles of molecular medicine. Springer; 2006. p. 893–904.
25. Prahoveanu E, Eşanu V. Immunomodulation with natural products. I. Effect of an aqueous extract of Raphanus sativus niger on experimental influenza infection in mice. Virologie. 1987;38(2):115–20.
26. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol. 2005;4(1):1–11.
27. Jassim SAA, Naji MA. Novel antiviral agents: a medicinal plant perspective. J Appl Microbiol. 2003;95(3):412–27.
28. Cáceres A, Girón LM, Alvarado SR, Torres MF. Screening of antimicrobial activity of plants popularly used in Guatemala for the treatment of dermatomucosal diseases. J Ethnopharmacol. 1987;20(3):223–37.
29. Smolinska U, Horbowicz M. Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. J Phytopathol. 1999;147(2):119–24.
30. Park J-H, Shin K-K, Hwang C-W. New antimicrobial activity from Korean radish seeds (Raphanus sativus L.). J Microbiol Biotechnol. 2001;11(2):337–41.