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ORIGINAL ARTICLE / ÖZGÜN MAKALE

CHOLINESTERASE AND TYROSINASE INHIBITORY ACTIVITY OF SUBCRITICAL WATER AND MICROWAVE EXTRACTS OF *RAPHANUS SATIVUS* L. 'RED MEAT' RADIX

RAPHANUS SATIVUS L. 'RED MEAT' KÖKLERİNİN SUBKRİTİK SU VE MİKRODALGA EKSTRELERİNİN KOLİNESTERAZ VE TİROZİNAZ İNHİBİTÖR AKTİVİTESİ

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ABSTRACT

Objective: *Cholinesterase and tyrosinase inhibitory activity potential of microwave-assisted and subcritical water extracts of Raphanus sativus L. Red Meat roots were investigated.*
Material and Method: *Total phenol, flavonoid and anthocyanin content of extracts from conventional solvent and advanced extraction systems were spectrophotometrically quantified. Acetylcholinesterase, butyrylcholinesterase and tyrosinase inhibitory activities were investigated with the calculation of the rate of absorbance change with kinetic readings.*
Result and Discussion: *The subcritical water extract was found to provide highest acetylcholinesterase, butyrylcholinesterase and tyrosinase inhibitory activities with an IC_{50} of 0.71 mg/ml, 2.13 mg/ml and 1.21 mg/ml, respectively. Consistent with the anaylsis of total phenol (27.57 mg GAE/g) and flavonoid (4.80 mg QE/g) contents, subcritical water extract of red meat radish can be considered as a potential source for products aiming enzyme inhibitory activity.*
Keywords: *Cholinesterase, microwave, Raphanus sativus, red meat radish, subcritical water, tyrosinase*

ÖZ

Amaç: *Raphanus sativus L. Red Meat köklerinin mikrodalga destekli ve subkritik su ekstrelerinin kolinesteraz ve tirozinaz inhibitör aktivite potansiyeli araştırılmıştır.*
Gereç ve Yöntem: *Geleneksel çözücü ve gelişmiş ekstraksiyon sistemlerinden elde edilen ekstrelerin toplam fenol, flavonoid ve antosiyanin içeriği spektrofotometrik olarak ölçülmüştür. Asetilkolinesteraz, bütirilkolinesteraz ve tirozinaz inhibitör aktiviteleri, kinetik okumalarla absorbans değişim hızının hesaplanmasıyla araştırılmıştır.*
Sonuç ve Tartışma: *Subkritik su ekstresinin sırasıyla 0.71 mg/ml, 2.13 mg/ml ve 1.21 mg/ml IC_{50} değerleri ile en yüksek asetilkolinesteraz, bütirilkolinesteraz ve tirozinaz inhibitör aktivitelerini sağladığı bulunmuştur. Toplam fenol (27.57 mg GAE/g) ve flavonoid (4.80 mg QE/g) içeriği analizi ile de tutarlı olarak, kırmızı etli turpun subkritik su ekstresi enzim inhibitör aktivite amacıyla üretilen ürünler için potansiyel bir kaynak olarak değerlendirilebilir.*
Anahtar Kelimeler: *Karpuz turpu, kolinesteraz, mikrodalga, Raphanus sativus, subkritik su, tirozinaz*

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INTRODUCTION

The sustainable use of natural sources for medicinal purposes requires the search for appropriate plant materials and extraction systems. Identification of plant sources that carry bioactives with therapeutic potential and are also easy to grow, store, transport and mass produce can be an effective approach towards sustainability goals. On the extraction technology side, systems with reduced solvent, energy, raw material and time consumption are valuable for both environmental and economic benefits.

Raphanus sativus L. 'Red Meat' (Brassicaceae) is an agricultural radish cultivar which has an inner tissue with red color due to anthocyanins. This cultivar has been called red meat radish, watermelon radish and also Chinese radish. Radish is widely grown and consumed throughout the world, especially in East Asia. Several cultivars have been produced with a wide variety of shape and color. Various parts of radish such as roots, leaves, stems and seeds have been used for medicinal purposes with laxative, stimulant, digestive, stomachic and decongestant aims. The main constituents are phenolic acids, flavonoids (including anthocyanins) and isothiocyanates. Various preparations of *Raphanus sativus* have been shown to exhibit antioxidant, antimicrobial and anticarcinogenic activities [1-3]. Traditionally, radish has been used for food and medicinal purposes. Radish can be consumed as cooked or raw as meal, mainly in salads. For treatment purposes, radish has been used in home remedies against cough, rheumatoid arthritis and and gallstones; in addition to jaundice, liver disease and gastric disorders in Unani, Greeko-Arab, Indian folk medicine, and kidney stones in Yemenite folk medicine [4]. In traditional Chinese medicine, it was mainly used as digestive aid, and for the treatment of diarrhea, dysentery, and cough. Radish was benefited for carminative, diuretic, expectorant, stomachic purposes in Korean and Indian medicine [5].

Advanced extraction techniques have been valuable in obtaining plant bioactives, as they provide a more feasible process, particularly for production purposes. Microwave systems have been emphasised as improved technologies for the extraction of herbal material because they can better protect thermolabile compounds and offer greater yields of active compounds with less time, energy and solvent consumption. Without the need for adding organic solvents, polarity adjustment can be achieved through subcritical water extraction. Subcritical water is one of the green extraction methods that aims to reduce the amount of raw materials, energy and organic solvents used, while increasing selectivity and efficiency and leaving no harmful residues [6,7]. There have been studies investigating the effect of the subcritical water and microwave assisted extractions of phenolic rich plants targeting enzyme inhibitory activities. For the subcritical water extraction of black mulberry fruits, 100°C-60 min.-2 ml/min-150 bar conditions were found to have the highest total phenol (46.71 mg GAE/g), flavonoid (7.34 mg QE/g) and anthocyanin (1.05 mg Cya3GluE) contents with an IC_{50} of 1.48 mg/ml for tyrosinase inhibitory activity [8]. The subcritical water extraction of red cabbage leaves was studied in the range of 120-160°C, where optimum conditions were determined to be 160°C-30 min.-10 solvent:solid ratio-200 bar conditions with total phenol (89.34 mg GAE/g), flavonoid (13.13 mg QE/g), anthocyanin (1.03 mg Cya3GluE/g) contents and exhibiting acetylcholinesterase (IC_{50}: 0.058 mg/ml), butyrylcholinesterase (IC_{50}: 0.215 mg/ml) and tyrosinase (IC_{50}: 0.86 mg/ml) inhibitory activities [6]. In the microwave extraction of black mulberry fruits at 500 watt-10 min.-1:10 solid:solvent ratio conditions, nearly 2 times more potent extract (IC_{50}: 1.44 mg/ml) than conventional solvent extracts (orbital shaker extract IC_{50}: 2.81 mg/ml, ultrasonic bath extract IC_{50}: 2.61 mg/ml) for tyrosinase inhibitory activity was achieved with improved total phenol (21.05 mg GAE/g), flavonoid (2.62 mg QE/g) and anthocyanin (13.28 mg Cya3GluE/g) contents [9]. The optimum conditions for microwave extraction of red cabbage leaves were determined to be 800 watt-4 min.-50% ethanol conditions with total phenol (36.14 mg GAE/g), flavonoid (3.41 mg QE/g) and anthocyanin (10.38 mg Cya3GluE/g) contents, while providing the highest tyrosinase inhibitory activity (IC_{50}: 5.03 mg/ml). The highest butyrylcholinesterase inhibitory activity (IC_{50}: 0.48 mg/ml) was exhibited by 600 watt-12 min.-100% ethanol condition extract, while acetylcholinesterase inhibition was found to be below 50% inhibition at 6.25 mg/ml [7].

Phenolic compounds have been shown to be important among bioactive substances due to their capability to exert a wide range of activities. It has been demonstrated that plant phenolics and extracts have the ability to inhibit certain enzymes which were linked with diseases [10-12]. Acetylcholinesterase and butyrylcholinesterase enzymes were shown to have role in Alzheimer's and

Parkinson’s diseases. Alzheimer’s disease is a complex neurodegenerative illness, and the progress is affected by various parameters like age and genetics. The degeneration of cholinergic neurons has been pointed out as the cause of cognitive impairment due to reduced cholinergic transmission. Inhibition of cholinesterase enzymes has been a treatment option to overcome this issue. While acetylcholinesterase activity has been mainly targeted, mechanisms to inhibit butyrylcholinesterase also present importance due to its discovered activity in neuronal lesions in Alzheimer’s disease [13]. Similar treatment has also been studied for the cognitive impairment in Parkinson’s disease, where the use of cholinesterase inhibitors was shown to provide significant improvement in clinical trials [14]. The critical role of tyrosinase enzyme has been demonstrated in the formation of hyperpigmentation due to excess accumulation of produced melanin and melanogenesis in melanoma. Therefore, research on natural bioactives that can inhibit tyrosinase activity has long been conducted [10].

As a source of phenolic compounds, investigation of extracts from red meat radish roots targeting acetylcholinesterase, butyrylcholinesterase (associated with cognitive impairment in Alzheimer’s and Parkinson’s disease) and tyrosinase (associated with hyperpigmentation and melanoma) activity may reveal beneficial effects and its medicinal potential.

In this study, the research of enzyme inhibitory potential of a plant source which could be appointed as a good candidate for above listed properties, using advanced extraction systems as microwave assisted and subcritical water extraction was aimed.

MATERIAL AND METHOD

Plant Material

Fresh roots of red meat radish (*Raphanus sativus* L. ‘Red Meat’) were obtained from an agricultural production site in Bayindir Tokatbasi village, Izmir, Turkiye. The roots were cleaned from any residual dirt, white outermost parts were peeled off and red innertissue was sliced for drying. A controlled airflow drying cabinet was used for drying the roots. All of the dried roots were stored at -24 °C until the experiments. Prior to the extractions, the plant material was ground to obtain a homogeneous batch. A voucher specimen was kept at IZEF Herbarium located in Ege University, Türkiye (IZEF-6716).

Extractions

Conventional solvent extractions with 70% ethanol and water were performed with ultrasonic bath (Isolab, Germany). Before extractions, dried roots were homogenized with a blender (Isolab, Germany). In glass flasks, 5 g ground roots were mixed with 100 mL extraction solvents. 0.1% TFA (trifluoroacetic acid) was added to maintain a pH under 3 for anthocyanin’s stability. Each round as 1 h was performed at 25°C in dark. After a total of three rounds, the filtrates were combined for evaporation with a rotary evaporator (Buchi, Switzerland). A vacuum concentrator system (Thermo Scientific, USA) and a lyophiliser (Labconco, USA) were used to totally dry the extracts. The samples were stored at -24°C during the experiments [7].

Microwave assisted extraction was performed with a closed vessel microwave system (Sineo, China). In each microwave vessel (2 vessels used) 2,5 g of ground dried roots were mixed with 50 mL extraction solvent and extracted under optimized conditions previously identified in an optimization study. The studied optimized conditions for extraction time, solvent composition and microwave power with the aim of higher bioactive content and enzyme inhibitory activity were as; 12 min-100% ethanol-600 watt, 4 min-50% ethanol-800 watt and 8 min-50% ethanol- 600 watt. Microwave assisted extractions were performed at 25°C. After the extractions, the filtrates were evaporated with the rotary evaporator. Complete dryness of the samples were ensured using the vacuum concentrator system and the lyophiliser. The samples were stored at -24°C during the experiments [7].

Subcritical water extraction was performed with a high pressure reactor system (Amar Equipments, India). The extractor was filled with 10g of ground roots and 100 ml of water. A previously determined optimized conditions of 160°C-30 min-10 solvent:solid ratio were used for subcritical water extraction. A batch extraction was performed at 200 bar. After the extraction, the filtrate was evaporated with the rotary evaporator. Complete dryness of the samples was ensured using the lyophiliser. The samples were stored at -24°C during the experiments [6].

Analysis of Bioactive Content

Total phenol, flavonoid and anthocyanin contents of the extracts were determined with spectrophotometry (BMG Labtech, Germany). 96 well microplate methods based on Folin-Ciocalteu, aluminum chloride and pH differentiation methods were used, respectively. Gallic acid and quercetin (1-250 µg/ml) were used as reference standards to establish the calibration curves. Absorbance measurements were performed at 760 nm and 415 nm for total and flavonoid contents, while 520 nm and 700 nm for total anthocyanin content. The results of triplicate analysis for total phenol, flavonoid and anthocyanin contents were expressed as mg gallic acid equivalent (GAE)/g extract, mg quercetin equivalent (QE)/g extract and mg cyanidin-3-glucoside equivalent (Cya3GluE)/g extract [7]. Statistical significance (p<0.05) of the results were determined with one-way ANOVA and post hoc comparison Tukey's HSD Test using IBM SPSS Statistics 29.0 software.

Enzyme Inhibitory Activity

Acetylcholinesterase, butyrylcholinesterase and tyrosinase inhibitory activities of the samples were determined with microplate assays. Previously developed and validated assays using inhibiton kinetic models (Michaelis-Menten, Lineweaver-Burk and Eadiee-Hofstee methods) were used. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates and galantamine as reference drug for cholinesterase inhibitory activity, whereas levodopa and kojic acid were used for tyrosinase inhibitory activity, respectively. Enzyme inhibitory activities were determined with kinetic readings taken with 30 sec intervals during incubation period (10 min for cholinesterase, 5 min for tyrosinase). The activity results were calculated based on the rate of absorbance change during incubation period against blank. The results of triplicate analysis were acquired to calculate IC_{50} values by linear regression analysis using GraphPad Prism 5 software [7].

RESULT AND DISCUSSION

The preparation of the homogeneous dried batch of the plant material was carried out with ten parallel samples. Each sample was weighed during the drying process and the moisture content was calculated. An average dry weight percentage of 9.50%±0.61 with a relative standard deviation of 6.45% was observed. The moisture content of the samples was determined as 91.50%, which was in agreement with the literature data [15].

Conventional solvent extracts (70% ethanol and water), microwave assisted extracts (12 min-100% ethanol-600 watt, 4 min-50% ethanol-800 watt and 8 min-50% ethanol- 600 watt) and subcritical water extracts of red meat radish were evaluated for bioactive content and enzyme inhibitory activity (Table 1). Among the solvent extracts, 70% ethanol (0.1% TFA) provided higher total phenol (10.87 mg GAE/g), flavonoid (0.92 mg QE/g) and anthocyanin (3.71 mg Cya3GluE/g) contents compared to water extract. In accordance with literature data, higher bioactive content was observed with aqueous ethanol with respect to the phenolic compounds [16]. An 80% methanol extract of red radish pulps was determined with a total phenol content of 41.17 mg GAE/g dry weight [3], while in another study the total phenol content of watermelon radish was shown to be 6.27 mg GAE/g dry weight [17]. Lower levels of total phenol content (6.07 mg GAE/g dry weight) obtained with 70% ethanol extraction could be related to variations in *Raphanus sativus* cultivars. The difference for total anthocyanin content was outstanding with 2.3 times, where 70% ethanol was also previously determined with enhanced capability for anthocyanin extraction [7]. Regarding the microwave assisted extraction, the highest total phenol content (5.21 mg GAE/g) was obtained with 8 min-50% ethanol-600 watt conditions. 12 min-100% ethanol-600 watt conditions provided the highest total flavonoid content (0.67 mg QE/g) while 4 min-50% ethanol-800 watt was found to be superior for total anthocyanin content (1.54 mg Cya3GluE/g). The results were found to be consistent with previous findings, as simultaneous increase in both extraction time and ethanol concentration had a positive effect on total phenol content [7]. However, in another study contrary results were observed. In the study with the black mulberry fruits, lowering the ethanol conc. to 35% with an extraction time of 10 min allowed the extraction with the highest total phenol content [9]. This situation may highlight the limited applicability of the direct use of different predefined optimisation parameters determined for subcritical water and microwave extractions, which can vary greatly due to the form of the studied plant material as fruit, leaf or root. The anthocyanin content of red meat radish (from USA) juice was quantified as 33.6 mg/100 g roots in a previous study [18]. The extraction of *Raphanus sativus* L. var. Crimson Gigant (from Mexico) was performed with 85% methanol (0.01% HCl), where the total anthocyanin content was determined as 17.36 mg Cya3GluE/g fresh weight [19]. In another study, the purified anthocyanin fraction of *Raphanus sativus* L. (from USA) was quantified as 154 mg Pel3GluE/100 g fresh weight for total anthocyanin content [20]. The total anthocyanin content of aqueous acetone (30:70) extract of red meat radish (from USA) was determined to be 53 mg Pel3GluE/100 g fresh weight [21]. Acidified hexane extract of red meat radish (a red flesh *Raphanus sativus* L. cultivar from China) was shown to yield 2.08 mg Pel3Glu/g fresh weight [22]. The research on methanol (0.1% HCl) extracts of twenty-four radish varieties from India exhibited a great variation in bioactive content; where anthocyanin content was found to vary as much as 36.16-fold (ranging from 0.46 to 16.6 mg Cya3GluE/100 g fresh weight) among the radishes tested [23]. The variations in anthocyanin content of radishes in different studies may be determined due to the extraction method applied, the quantification reference used (quantification based on cyanidin-3-glucoside or pelargonidin-3-glucoside), the use of radishes from different cultivars, genotypic and environmental factors [21-23]. Based on the crude extracts, 70% ethanol ultrasonic extraction outperformed both microwave and subcritical water extractions for total anthocyanin content. The microwave extraction (1.54 mg Cya3GluE/g extract, 0.23 mg Cya3GluE/g dry weight, 2.2 mg Cya3GluE/100 g fresh weight) was found with 2.4 times lower anthocyanin content compared to conventional solvent extraction (3.71 mg Cya3GluE/g extract, 2.07 mg Cya3GluE/g dry weight, 19 mg Cya3GluE/100 g fresh weight). With the studied extraction parameters, regarding the extraction of anthocyanins, selective and exhaustive extraction capability of the microwave and subcritical water extractions were determined to be limited. The highest values for the quantified bioactive contents were obtained with subcritical water extraction as 27.57 mg GAE/g and 4.80 mg QE/g for total phenol and flavonoid content, respectively. No anthocyanin content was able to be extracted with the studied parameters from red meat radish roots. Previous investigations on other anthocyanin rich sources as black mulberry fruits [8] and red cabbage leaves [6] had yielded 0.61 mg Cya3GluE/g and 1.03 mg Cya3GluE/g total anthocyanin content at 120°C-60 min-150 bar and 160°C-30 min-200 bar extractions, respectively. Therefore, 160°C can be considered as the overthreshold condition for the extraction of red meat radish anthocyanins, where reduced temperature values of around 120°C can be targeted for the optimization of subcritical water extraction of red meat radish roots in future studies. Both in the previous studies, increasing the temperature had a dramatically improving effect on total phenol and flavonoid contents of the extracts. Similar results were obtained with the subcritical water extraction of red meat radish, where 160°C-30 min-200 bar extract possessed 27.57 mg GAE/g and 4.80 mg QE/g total phenol and flavonoid contents, respectively. When the conventional solvent and advanced extraction systems (microwave and subcritical water) were compared, the subcritical water extract was found to provide the extract with the most rich bioactive content for total phenol and flavonoid contents, while the highest anthocyanin amounts were obtained with 70% ethanol extraction (Figure 1). The use of relatively high microwave power as 600 and 800 watts could be the reason for the limited ability for the extraction of red radish anthocyanins, where microwave extraction of red radish roots might better be more effective as studied with lower power values for anthocyanin extraction.

Subcritical water extract was found to provide an IC_{50} of 0.71 mg/ml and 2.13 mg/ml for acetylcholinesterase and butyrylcholinesterase inhibitory activity, respectively. IC_{50} values for cholinesterase inhibitory activity of conventional solvent and microwave assisted extracts could not be determined as 50% inhibition was not exceeded at the starting concentration of 1.50 mg/ml. For tyrosinase inhibitory activity, the strongest activity (IC_{50}: 1.21 mg/ml) was found in the subcritical water extract, similar to the cholinesterase inhibition results. Microwave II extract with the extraction conditions of 4 min-50% ethanol-800 watt provided the highest butyrylcholinesterase inhibitory activity among the microwave extracts studied. On the side of the conventional solvent extracts, IC_{50} of 70% ethanol extract for butyrylcholinesterase inhibitory activity was found to be 1.88 mg/ml. Compared with reference drugs as galantamin (IC_{50}: 0.23 µg/ml and 2.62 µg/ml) and kojic acid (IC_{50}: 4.82 µg/ml), all extracts from conventional solvent, microwave assisted, and subcritical water systems possessed remarkably weaker activity in the crude form. Among the extracts, subcritical water was noted with greater potency both for cholinesterase and tyrosinase inhibitory activity. In previous studies similar results were observed as 160°C-30 min-10 solvent:solid ratio extract was found to possess the highest acetylcholinesterase and butyrylcholinesterase inhibitory activity in red cabbage extracts [6]. Tyrosinase inhibitory activity of different radish varieties were determined with an IC_{50} of 3.09 mg/ml and 9.62 mg/ml for juice and methanol extracts of Thai radish [24], and 42% inhibition at 2 mg/ml dose for *Raphanus sativus* var. *caudatus* [25], respectively. Tyrosinase inhibition by ethanol extract of *Raphanus sativus* var. *longipinnatus* was shown to be 42.85% at 1.67 mg/ml dose [26]. In comparison with the literature, both subcritical water (IC_{50}: 1.21 mg/ml), microwave assisted (IC_{50}: 2.20 mg/ml) and conventional solvent (IC_{50}: 1.88 mg/ml) extracts of red meat radish were found to have greater potency for tyrosinase inhibitory activity.

Table 1. Comparison of microwave and subcritical water extracts of red meat radish with conventional solvent extracts

Microwave and Subcritical Water Extracts vs Conventional Solvent Extracts	Temp (°C)	Time (min)	Solvent: solid ratio	Ethanol conc. (%)	Power (watt)	TPC* (mg GAE/g)	TFC* (mg QE/g)	TAC* (mg Cya3 GluE/g)	AchEInh (IC_{50}, mg/ml)	BuChEInh (IC_{50}, mg/ml)	TyroInh (IC_{50}, mg/ml)
Microwave I	25	12	20	100%	600	4.51±0.12[a,b]	0.67±0.07[a,b]	0.82±0.04[a]	ND	ND	ND
Microwave II	25	4	20	50%	800	3.89±0.06[a]	0.61±0.09[a,b]	1.54±0.10[b]	ND	ND	2.20±0.09[a]
Microwave III	25	8	20	50%	600	5.21±0.38[b]	0.23±0.02[a]	1.08±0.10[a]	ND	ND	ND
Subcritical Water	160	30	10	0%	NA	27.57±1.09[c]	4.80±0.34[c]	-	0.71±0.04	2.13±0.18	1.21±0.04[b]
70% Ethanol (0.1% TFA)	25	180	60	70%	NA	10.87±0.10[d]	0.92±0.01[b]	3.71±0.17[c]	ND	ND	1.88±0.16[c]
Water (0.1% TFA)	25	180	60	0%	NA	8.83±0.07[e]	0.46±0.01[a,b]	1.62±0.01[b]	ND	ND	ND

$p<0.05$ (Different letters in column indicate statistical significance); *: mg/g extract ± SD; NA: Not applied; -: Not detected
ND: Not determined due to 50% inhibition could not be exceeded at 1.50 mg/ml concentration; TPC: total phenol content; TFC: Total flavonoid content; TAC: total anthocyanin content; GAE: gallic acid equivalent; QE: quercetin equivalent; Cya3GluE: cyanidin-3-glucoside equivalent; AChEInh: acetylcholinesterase inhibitory activity; BuChEInh: butyrylcholinesterase inhibitory activity; TyroInh: tyrosinase inhibitory activity

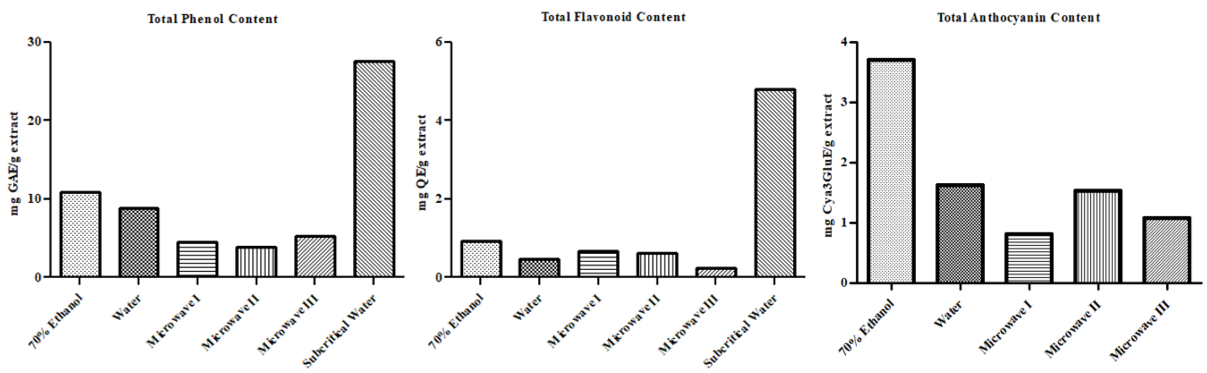

Figure 1. Comparison of bioactive content of red meat radish's conventional solvent, microwave assisted and subcritical water extracts (The statistical significance of the results are presented with Table 1)

Considering the content-activity relationship, the subcritical water extract with higher total phenol (2.7-fold) and flavonoid (5.3-fold) content was found to exhibit the most potent cholinesterase and tyrosinase inhibitory activities, where the activity results could be attributed to its rich phenolic composition. 70% ethanol extract was determined with a remarkable tyrosinase inhibitory activity among the tested samples, while it was identified with the highest total anthocyanin content (3.71 mg Cya3GlueE/g) (Table 1). Identical results were also obtained for the microwave extracts. Elevated total anthocyanin content (Microwave II extract) could be related to a higher tyrosinase inhibition. Similar to previous studies, the strongest tyrosinase inhibition was observed with the anthocyanin rich extracts in microwave extracts of black mulberry fruits [9] and red cabbage leaves [7]. Although the subcritical water extract was not able to provide anthocyanin content, improved tyrosinase inhibitory activity could be attibuted to its dramatically increased total phenol and flavonoid amounts, which was also consistent with literature data [6,8].

General advantages of microwave extraction have been denoted as the protection of active compounds due to shortened process duration, while consuming less energy and extraction solvent [7]. With the microwave extraction of red meat radish, 3 times lowered solvent amounts and dramatically reduced extraction time (180 min. vs 4 min.) was used. However, compared to conventional extractions, no improvement was observed for the extraction of targeted bioactive content (Table 1). Microwave extraction was able to yield a total anthocyanin content of 1.54 mg Cya3GluE/g using a 50% ethanol solvent system. Both microwave and conventional solvent extracts were unable to possess remarkable acetylcholinesterase and butyrylcholinesterase inhibitory activities (<50% inhibition at 1.50 mg/ml), while tyrosinase inhibition was observed with 800 watt-4 min.-20 solvent:solid ratio (IC_{50}: 2.20 mg/ml) and 70% ethanol ultrasonic (IC_{50}: 1.88 mg/ml) extracts.

Subcritical water has emerged as a green extraction technique with the advantage of eliminating the use of organic solvents by allowing the polarity of the extraction solvent to be regulated in the subcritical phase of water. In addition to the highlighted advantage of being free of toxic and harmful residues to nature and humans, the ability to improve selectivity for bioactive compounds is important [6]. Without the need to use any organic solvent, subcritical water extraction was found to possess the highest total phenol (27.57 mg GAE/g) and flavonoid (4.80 mg QE/g) contents for the extraction of red meat radish (Table 1). Consistent with the bioactive composition, the highest enzyme inhibitory activity was determined with the subcritical water extract for all studied enzymes. However, the subcritical water system was found to be incapable of extracting the red meat radish anthocyanins with the studied extraction parameters.

The main drawbacks for both subcritical water and microwave extractions could be pointed out as; higher initial cost of setup for high-tech equipment, need for training and experience to operate and maintain the extraction systems, and limited applicability of the discovered optimum extraction parameters in literature directly to the investigation of new plant materials, as each plant material may require a novel optimization study to possess the highest potential for bioactive content and targeted activity.

This is the first study comparatively revealing cholinesterase and tyrosinase inhibitory potential of subcritical water and microwave assisted extracts of red meat radish. In the pursuit of sustainability goals, red meat radish can be considered as a potential source of phenolic compounds with enzyme inhibitory activity through the extraction of advanced techniques. Further studies are planned on the fractionation and isolation of the subcritical water extract of red meat radish.

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AUTHOR CONTRIBUTIONS

Concept: H.K.; Design: H.K.; Control: H.K.; Sources: H.K.; Materials: H.K.; Data Collection and/or Processing: H.K.; Analysis and/or Interpretation: H.K.; Literature Review: H.K.; Manuscript Writing: H.K.; Critical Review: H.K.; Other: -

CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

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