

Study of antioxidant properties of alcoholic and aqueous extracts of radish and carrot seeds

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Extract

The current study aimed to evaluate and compare the total phenolic and total flavonoid content in carrot and radish seed extracts using different solvents (water and ethanol), as well as to determine their antioxidant efficacy through DPPH and ferrous ion binding (Fe^{2+}) tests. The study also sought to clarify the effect of plant species and solvent type on the efficiency of extracting bioactive compounds, and to demonstrate the relationship between the chemical composition of the extracts and their antioxidant activity, thus highlighting the potential applications of carrot and radish seeds as natural sources of antioxidants. The results of the current study showed a clear and significant difference in the total phenolic and total flavonoid content, as well as in antioxidant activity, between carrot and radish seed extracts depending on the plant species and the solvent used. The alcoholic extract of carrot seeds recorded the highest concentration of phenols at 3870.0 mg/g, compared to all other treatments, were followed by aqueous carrot extract (2150.0 mg/g). Then alcoholic radish seed extract (2350.0 mg/g). Meanwhile, the extract of water radish seeds recorded the lowest values (1420.0 mg/g). This superiority reflects the high efficiency of ethanol in extracting phenolic and flavonoid compounds of a polar and subpolar nature, which are among the most important compounds responsible for antioxidant activity. The results of the ferrous ion binding test showed (Fe^{2+}) There are significant differences between the extracts, as the alcoholic extracts clearly outperformed their aqueous counterparts, and the alcoholic carrot seed extract recorded the highest ability to bind Fe^{2+} (27.60%). This was followed by an alcoholic extract of radish seeds (21.33%). While aqueous extracts showed a lower ability to form complexes with iron ions (14.90-12.60%). This superiority is attributed to the high content of phenolic compounds and flavonoids in the alcoholic extracts, which are capable of inhibiting the catalytic activity of ferrous ions in Fenton reactions, thus reducing free radical formation and limiting lipid peroxidation. The DPPH assay results also showed a clear positive correlation between the concentration of the extracts and the degree of free radical inhibition in all treatments, with the alcoholic extracts significantly outperforming the aqueous extracts. The alcoholic carrot seed extract achieved the highest inhibition rates across all studied concentrations, followed by the alcoholic radish seed extract, then the aqueous carrot extract, and finally the aqueous radish seed extract. This ranking reflects the richness of the alcoholic extracts in compounds capable of donating hydrogen or electrons to free radicals and converting them into more stable compounds, particularly phenols, flavonoids, and carotenoids.

Introduction

Oxidation is one of the most important challenges facing food quality and safety, especially animal products such as fish, as oxidation reactions lead to a deterioration in sensory qualities and nutritional value. (1) In addition to forming byproducts harmful to health. The formation of free radicals and their reaction with lipids and proteins are among the

main factors responsible for this phenomenon (2). Where transition metal ions, especially ferrous ions, play a role (Fe^{2+}) plays a pivotal role in catalyzing oxidation reactions through Fenton reactions, which lead to the generation of reactive oxygen species. There is growing global interest in finding safe and effective natural antioxidants that can

replace synthetic compounds used in food preservation.(3).Phenolic compounds, particularly total phenols and flavonoids, are among the most important and highly effective natural antioxidants, as they have the ability to inhibit oxidation through several mechanisms.(4)This includes donating hydrogen atoms or electrons to free radicals, inhibiting their activity, and their ability to form stable complexes with transition metal ions such as ferrous ions, thus limiting their catalytic role in oxidation reactions.Numerous studies have shown that the effectiveness of these compounds is closely linked to the plant species and the plant part used, as well as the extraction method and solvent. Carrot seeds (*Daucus carota* L.) and radish seeds (*Raphanus sativus* L.) are promising plant sources of phenolic compounds and flavonoids.(5)Because it contains bioactive compounds such as phenolic acids, flavonoids, coumarins, carotenoids, and glucosinolates, which together contribute to enhancing antioxidant activity.However, the efficiency of extracting these compounds varies depending on the solvent used. Ethanol is one of the most common solvents for extracting phenolic compounds due to its ability to dissolve compounds of a polar and subpolar nature, compared to aqueous solvents whose effectiveness is limited to extracting some water-soluble compounds. Several laboratory tests are used to evaluate the antioxidant activity of plant extracts, most notably the testDPPH, which measures the ability of the active compounds to inhibit free radicals, and the ferrous ion binding capacity (Fe^{2+}) test, which reflects the ability of the extracts to inhibit oxidation by disrupting the role of transition metals, provide accurate scientific indicators of the efficiency of the extracts as natural antioxidants and their potential for use in food applications.As a natural preservative, an alternative to artificial preservatives (6)

Materials and methods

Total Phenols

The total phenolic content of radish and carrot seed extracts was estimated according

to the method used by(8) 2.5 ml of Follen's reagent was taken and added to 0.5 ml of the prepared extracts equivalent to (1 mg/ml). The mixture was then left for 10 minutes at room temperature (25°C). After that, 2 ml of sodium carbonate with a concentration of 7.5% was added, and the mixture was left to react for 30 minutes. The absorbance was then measured at a wavelength of 760 nm using a spectrophotometer

Flavonoids Determination of Total The amount of flavonoids in aqueous and alcoholic extracts of radish and carrot seeds was estimated using the method described by (8)This includes taking 2 ml of aqueous aluminum chloride. $AlCl_3 \cdot 5H_2O$ prepared at a concentration of 2% in 100 ml of ethanol, then 2 ml of extracts were added, then the mixture was shaken well and left for one hour at room temperature 25°C, then the absorbance was measured at a wavelength of 415 nm

Ferrous ion binding abilityChelating of Ferrous Ion

The ability of aqueous and alcoholic extracts of radish and carrot to bind ferrous ions was estimated according to the method described in (9)Then, the ability of the ferrous ion to bind was measured using the following equation :

Ferrous ion binding capacity = $[1 - \text{Model absorbance} / \text{Control sample absorbance}] \times 100\%$

Scavenging of Free Radical Abili

The antioxidant activity of radish and carrot seed extracts was estimated using an indicatorDPPH was used to estimate the effectiveness of free radical inhibition, as described in the method previously described (10). The synthetic antioxidant (BHT) was used for comparison. This involved taking 1 ml of the prepared extracts and adding them to 3.7 ml of methanol, along with the addition of 0.1 ml of ferrous chloride at a concentration of 2 mM and 0.2 ml of ferrosilicon (a substance added to determine the end result of the reaction) at a concentration of 5 mM. The mixture was then mixed and left for 10

minutes at room temperature (25°C). The absorbance was then measured at a wavelength of 562 nm. The control sample was prepared in the same way, except for the addition of the alcoholic, aqueous, and oily extracts. The ferrous ion binding capacity was then measured, and the percentage of free radical inhibition activity was calculated using the following equation: Absorption reading of the model

Free radical capture capability == $[1 - \text{Model absorbance} / \text{Control sample absorbance}] \times 100\%$

Results and discussion

Estimation of the total flavonoid and phenol content in radish and carrot seed extracts

Figure (1) shows the percentage of flavonoid and phenol content in the seed extracts. The alcoholic carrot extract recorded the highest concentration of total phenols, with an average of 3870 mg per 100 g of dry matter, followed by the aqueous carrot extract with an average of 2150 mg, then the alcoholic radish seed extract with an average of 2350 mg, and finally the aqueous radish extract, which recorded the lowest value with an average of 1420 mg. As for total flavonoids, the same treatments maintained the same order of values, with the alcoholic carrot extract also being the most abundant, with an average of 1890 mg per 100 g, followed by the aqueous carrot seeds with an average of 980 mg, then the alcoholic radish with an average of 1150 mg, and finally the aqueous radish seeds, which recorded the lowest values with an average of 630 mg. This remarkable superiority of the alcoholic carrot seed extract is attributed to the fact that ethanol has a high efficiency in dissolving polar and subpolar phenolic compounds, which include coumarins, flavones, phenols, and chlorogenic acid. Anthocyanins and many other active compounds are concentrated in islet tissues,

especially the cortex and parenchyma cells, as indicated by the study.(11)The alcoholic extract of carrots contains the highest concentration of bioactive compounds compared to the aqueous extract, which explains the current results and supports the hypothesis that the type of solvent plays a pivotal role in the extraction of active phenols. Radishes, on the other hand, showed a relatively lower content in both the aqueous and alcoholic forms. This is attributed to the lower natural concentrations of phenolic compounds in radish roots compared to carrots, in addition to the fact that the composition of phenolic compounds in radishes differs, being more concentrated in the seeds and leaves than in the main root, as a study concluded.(12)However, the alcoholic extract of radish contains phenolic compounds in good proportions, though still less than that of carrots, which is consistent with the results of this study. When comparing these results with those reported in studies that used carrot and radish seeds, we find that carrot seeds contain approximately 2760 mg of phenols and 1030 mg of flavonoids per 100 g, according to what was mentioned. (13)While white radish seeds recorded approximately 1820 mg of phenols and 740 mg of flavonoids, as reported in a study (14)It is clear that the alcoholic carrot extract is superior in terms of efficiency and concentration to both the seed and radish extracts. This confirms that the type of plant part used and the solvent are important factors in determining the efficiency of extraction and the value of the biological content of the extracts. Therefore, the alcoholic carrot extract at a concentration of 1500 mg/L can be considered the most effective as a natural antioxidant in food and fish preservation applications, especially when compared to the other types of extracts used in this research.

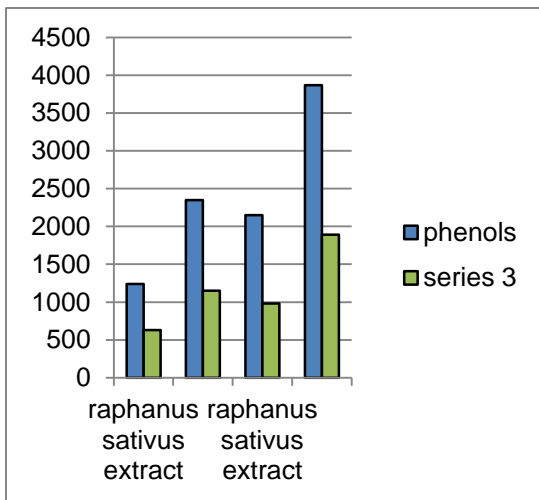


Figure (1) shows the total phenol and flavonoid content of the alcoholic and aqueous extracts of radish and carrot seeds.

The ability of radish and carrot seed extracts to bind ferrous ions

Figure (2) illustrates the results of the statistical analysis, showing significant differences between the extract treatments in their ability to bind ferrous ions. Fe^{2+} , where the alcoholic carrot extract outperformed all other studied concentrations, recording the

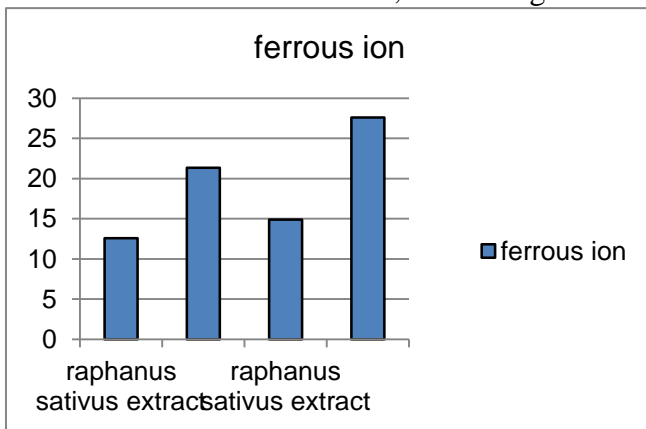


Figure 2 shows the ability of plant extracts to bind ferrous ions

Examine DPPH For plant extracts seeds Radishes and carrots Water and alcohol

The results in Figure (3) show that the extract of water radish seeds exhibited antioxidant activity starting from 23.56% at a concentration of 0.12 mg/ml and gradually increasing to 63.05% at a concentration of 1 mg/ml. This indicates that increasing the concentration of the seed extract enhances the inhibitory capacity against free radicals, and

highest binding percentage at 27.60%, followed by the alcoholic radish seed extract at 21.33%. Aqueous extracts, however, showed less effectiveness, with the aqueous carrot seed extract recording 14.90% and the aqueous radish seed extract having the lowest binding capacity at 12.60%. This superiority of the alcoholic extracts is attributed to their efficiency in extracting nonpolar

this is shown within the limits of the lowest significant difference. The calculated LSD of 0.67 confirms the existence of significant differences between the various concentrations. Radish seed extracts contain glucosinolates that contribute moderately to antioxidant activity, particularly in aqueous solvents. However, the alcoholic radish seed extract showed a clear advantage in inhibitory activity, starting at 20.54% at a concentration of 0.12 mg/ml and increasing to 66.24% at the

highest concentration of 1 mg/ml, with an LSD value of 1.01. This indicates that the difference between concentrations is not only large but also significant, reflecting the efficiency of alcohol in extracting active phenolic and flavonoid compounds. This was further confirmed by the study.(18)The study indicated that alcohol is a moderately polar solvent capable of extracting active compounds with a high effect in combating free radicals. The aqueous extract of carrot seeds showed moderate activity, starting at 23.24% and gradually increasing to 56.21% at a concentration of 1 mg/ml. This trend is similar to that observed in the aqueous extract of radish, but to a lesser extent. This suggests that some antioxidant compounds, such as ascorbic acid and water-soluble carotenoids, are responsible for this activity, and demonstrates the value ofThe LSD concentration of 1.16 indicates that these differences between the four concentrations were statistically significant.(19)Carrots contain carotenoids with moderate activity in the aqueous state. Regarding the alcoholic carrot extract, it achieved the highest activity

among all extracts, starting at 52.07% at a concentration of 0.12 mg/ml and peaking at 78.34% at a concentration of 1 mg/ml. This indicates that alcoholic solvents are efficient in extracting active compounds such as flavonoids and carotenoids, and this trend reinforces the value ofThe calculated LSD values, showing that the increase in inhibition was significant across different concentrations, were supported by a study (20)The study showed that alcoholic carrot extracts contain high concentrations of effective antioxidants such as phenols and carotenoids, which contribute significantly to the inactivation of free radicals when using organic solvents. The results generally indicate that the alcoholic carrot extract is the most effective in inhibition, followed by the alcoholic radish, then the water carrot, and finally the water radish. This demonstrates the effect of the plant type and the method of extraction on the effectiveness of antioxidants, which is consistent with recent studies that compare types of extracts, their solvents, and their biological effectiveness.

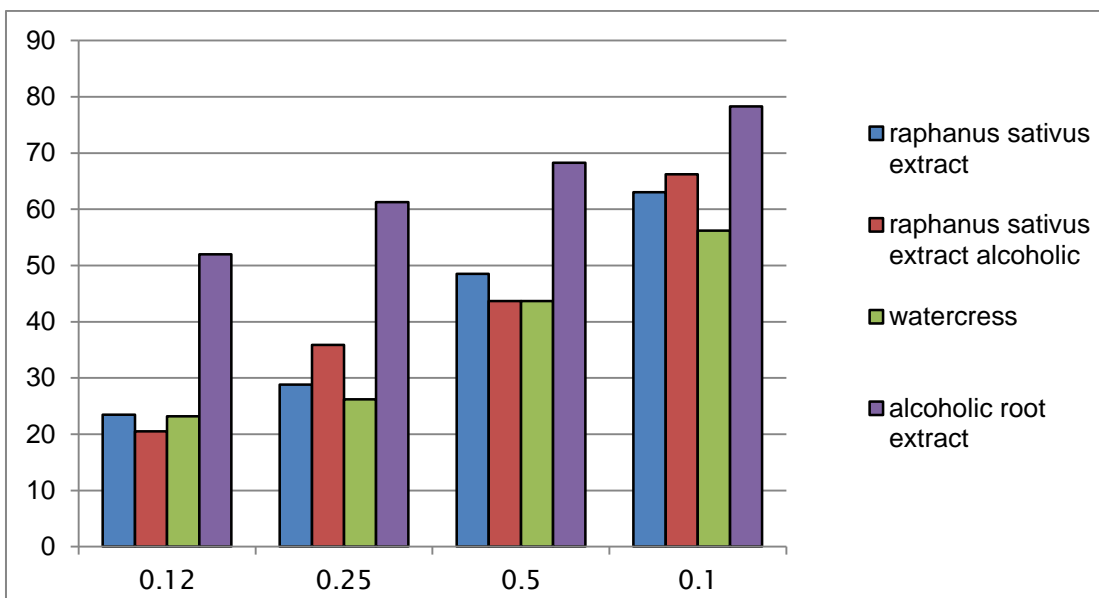


Figure 3 shows the effect DPPH for aqueous and alcoholic extract of radish and carrot

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