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Effect of Extractant and Temperature on Phenolic Compounds and Antioxidant Activity of Selected Spices

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Abstract

Ten spices marketed in Jordan, (Syzygium aromaticum L., Coriadrum sativum L., Cuminum cyminum L., Zinaiber officinale Rosc., Elettaria cardamomum, Curcuma longa, Rhus corigria L., Cinnamomum zeylanicum Blume, Foeniculum vulgare Mill and Laurus nobilis L.) were investigated for their phenolic compounds and antioxidant activity. The influence of different extractants (methanol, ethanol and acetone) at different temperatures (20, 40 and 60°C) was examined. Results showed at 60°C using methanol, cloves had the highest level of total phenolics (781.0 mg GAE/100g using acetone). At 40°C, sumac and cloves had the highest amounts of total phenolics (343.9 mg/100g and 342 mg GAE/100g respectively). At 20°C, cloves continued to have the highest amount of total phenolics (394.7 mg/100g) using methanol as extactant. Ethanol as extractant, cloves gave the highest level of phenolics (548 mg GAE/100g, 493.4 mg GAE/100g) at 60°C, while at 20°C cloves and sumac showed the highest concentrations of phenolics (350.8 mg GAE/100g and 342.8 mg GAE/100g respectively). Acetone as extractant at 60°C, cloves had the highest levels of phenolics (781 mg GAE/100g) while at 40°C and at 20°C, sumac contributed the highest levels of total phenolics (583.2 mg GAE/100g and 754.5 mg GAE/100g). The total phenolics concentration varied significantly among the spices. Their values varied according to the extractant and extracting temperature. IC₅₀ (radical scavenging activity) reflecting the antioxidant activity was presented. Results showed that cloves had the highest antioxidant activity while the cinnamon, turmeric and sumac had a appreciable level of antioxidant activity. Green cardamom and coriander had the lowest antioxidant activity. Antioxidant activity was positively correlated with total phenolic compounds content of the investigated spices.

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Keywords

Phenolic Compounds, Antioxidant Activity, Spices, Solvents, Extractant, Temperature

1. Introduction

Spices are one class of the aromatic plants; they are mainly present in the tropical provinces; the spices could be either as seeds, flowers or leaves [1]. On the other hand, herbs are fragrant and non-woody plants in which they are used in flavoring food dishes; the herbs could be leaves, stems or seeds. These additives are supplemented in small amounts to the food to enhance the flavor. Nutritionally, the spices and herbs are significant in reducing the peroxidation of lipids, which is the change (off-flavor) in the nature and the chemical composition of lipids during the processing, preservation and the final preparation of foods [2]. Spices prevent the lipid oxidation process due to the presence of natural antioxidants [3] [4].

The impact of antioxidants in spices exceeds the prevention of the oxidation process to influence the health of humans [5]. Several medicinal studies proved that they promote health and the immunity of humans; the existence of phytochemicals such as the phenolic compounds in plants, protect humans from cancers, depression, inflammations as well as their significance in treating heart diseases [5]. The phytochemicals are usually used to refer to the compounds found in plants that are not required for normal functioning of the body. Formally, the phytochemicals are defined as bioactive substances from plants which have beneficial effect on human health [6] [7].

Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases [8]. The consumption of fruits and vegetables is associated with lower risk of cancer, heart disease, hypertension and stroke. This has been attributed to the presence of phytochemicals and antioxidants in food. These phytochemicals may be present in small amounts but are very important to the health of consumers.

The antioxidant property in many plants is related to the presence of phenolic compounds. Nutritionally, these compounds are responsible for increasing the shelf life of foods as well as slowing the lipid, protein and enzymatic oxidation. In addition, phenolic compounds reduce the rancidity development, which prevent off-flavor of foods [9]. Antioxidants are specific additives that inhibit oxidation through reacting with free radicals; hence; forming inactive products. Antioxidants are any substance that prevents the rancidity or other flavor decline. The types of antioxidants are the natural and synthetic antioxidants; both of these additives include phenolic compounds [10]. Extraction of phenolic compounds using variety of solvent systems, time and temperature combination showed that the levels of extracted phenolics always depend on extractant, temperature, method of phenol extraction and their interaction.

This work aims to determining the levels of phenolic compounds in several spices marketed in Jordan. The project also will evaluate the influence of different extraction solvents and extraction temperatures on the concentrations of these compounds.

2. Materials and Methods

2.1. Spices Samples

About 500 g of the investigated spices (**Table 1**) were purchased from a local market, in Irbid city in Jordan. All the spices were purchased twice with two months time interval. Their scientific and local names were obtained from different references. All the purchased spices were pulverized to a fine powder using a laboratory mill to pass a 0.5 mm sieve and kept at 4°C in Ziploc plastic bags until time of analysis.

2.2. Extract Preparation

The extractants of the investigated spices were prepared by using three different solvents (methanol, ethanol and acetone) to investigate the effect of solvent on phenolic compounds levels at three different temperatures (20, 40 and 60°C) for each extractant.

One gram of each spice was weighed out and extracted with 50 ml of methanol. Extraction was carried out

Table 1. The investigated spices marketed in Jordan.

English Name	Scientific Name	Plant Family	Used Part
Cloves	Syzygium aromaticum L.	Myrtaceae	Ripe fruits
Coriander	Coriadrum sativum L.	Apiaceae	Fruit (seeds) leaves
Cumin	Cuminum cyminum L.	Apiaceae	Fruits (seeds)
Ginger	Zingiber officinale Rosc.	Zingiberaceae	Fleshy rhizome
Green Cardamom	Elettaria cardamomum	Zingiberaceae	Seeds
Turmeric	Curcuma longa L.	Zingiberaceae	Rhizome
Sumac	Rhus coriaria L.	Anacardiaceae	Dried fruits
Cinnamon	Cinnamomum zeylanicum Blume	Lauraceae	Stem bark
Sweet Cumin, (Fennel)	Foeniculum vulgare Mill	Apiaceae	Seeds, leaves
Sweet Laurel, (Sweet Bay leaf)	Laurus nobilis L.	Lauraceae	Leaves

under stirring for 60 minutes at 60°C. Each one was filtered using filter paper into a 50 ml volumetric flask, the volume was made up to mark using the same extractant and kept in the dark at refrigerated temperature until the time of analysis.

2.3. Phenolic Compounds Determination

The Folin-Ciocalteu assay [11] was used to determine the total content of phenolic compounds. Folin-Ciocalteu reagent is not specific to detect all phenolic groups found in the extracts. The total phenolic compounds were assayed calorimetrically:, 0.2 ml of spice extract (two replicates) were transferred into a test tube, then mixed with 0.4 ml of 10% diluted Folin-Ciocalteu reagent, after three minutes 0.8 ml of a 10% solution of sodium carbonate as a buffer was added and mixed well, all the tubes were allowed to stand for one hour at room temperature. The absorbance was measured at 725 nm using the spectrophotometer (CE CELL, model 1020). A mixture of reagents without the sample was used as a blank. The phenolic compounds content was expressed as gallic acid equivalents (mg GAE/100g) on dry weight basis; the gallic acid was used to prepare a calibration curve. Gallic acid standard was used to prepare the standard curve as follow; 200 mg of gallic acid were dissolved in 20 ml of distilled water, 0, 10, 25, 50, 100, 200 and 400 ppm concentrations, were prepared, all reagents wereadded as above and the absorbance was read at 725 nm.

2.4. Effect of Extraction Solvent

Organic solvents; methanol, ethanol and acetone were used to extract phenolic compounds from the spices. One gram of each spice was weighed out and subjected to extraction. Extraction was done under stirring while holding one extraction temperature: 20, 40 and 60°C for one hour at constant level. Each one was filtered using filter paper into a 50 ml volumetric flask, kept in the dark at refrigerated temperature until the time of analysis.

2.5. Effect of Extraction Temperature

Approximately, one gram of each spice was subjected to extraction at 20°C, 40°C and 60°C. Extraction was done under stirring while using one extraction solvent; methanol, ethanol and acetone. The extraction time was 60 minutes. Each one was filtered using filter paper into a 50 ml volumetric flask, kept in the dark at refrigerated temperature until the time of analysis.

2.6. Radical DPPH Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl)radical scavenging was estimated according to the method of Mättaus [12]. The scavenging effect of DPPH was followed by monitoring the decrease in absorbance at 515 nm that occurs due to the reduction by the antioxidants or reaction with a radical species. Approximately, 2 g (two rep-

licates) of each spice sample were extracted under stirring with 50 ml methanol for 60 minutes, at 60° C. Different levels of methanol extracts (10, 25, 50, 100, and 200 µg/ml) of each spice were reacted with 0.2 ml of DPPH (50 mg of DPPH in 100 ml methanol). The mixture was brought to a total volume of 4.0 ml with the extracting solvent. The mixture was mixed thoroughly and allowed to stand in the dark for 30 minutes. Absorbance (A) was read at 515 nm, against the blank. IC₅₀ (the efficient concentration of spice extract in mg/ml required to decrease initial DPPH radical concentration by 50%) was obtained by interpolation from linear regression analysis.

3. Statistical Analysis

The collected data were statistically analyzed using analysis of variance (ANOVA), using the general linear model procedure of SAS institute [13]. Data for each test were analyzed as a Complete Randomized Block Design (CRBD). Differences among treatment means were separated using the Least Significant Differences (LSD) at $p \le 0.05$.

4. Results and Discussion

4.1. Effect of Extraction Solvent

Tables 2-4 show data on total phenolic compounds content extracted by different solvents; methanol, ethanol and acetone at 60, 40 and 20°C. Data shows that total phenolic compounds concentrations had been affected significantly by the extractant used and temperature employed. Total phenolic concentrations ranged between 6.1 (coriander) and 305.5 mg GAE/100g (cloves) when the methanol was used as extractant at 60°C. All other spices found to have intermediate values (**Table 2**).

When ethanol was used, phenolic concentrations showed higher values than the methanolic extractant. Cloves, sumac and cinnamon had the highest values (548.0, 317.4 and 214.5 mg GAE/100g, respectively), whereas; green cardamom, coriander, cumin and ginger had the lowest concentrations (8.4, 13.8, 20.8 and 23.2 mg GAE/100g, respectively).

The phenolic compound levels in cloves found to be the highest as compared with values for other spices when acetone was used as extractant (781.0 mg GAE/100g) followed by sumac, cinnamon, turmeric and ginger (725.1, 293.4, 164.4 and 33.5 mg GAE/100g, respectively). Whereas, green cardamom, coriander and sweet laurel had the lowest values (4.5, 5.6 and 9.1 mg GAE/100g respectively).

Phenolic compounds concentrations extracted by methanol, ethanol and acetone showed a significant variation among the investigated spices, except for cumin and sweet cumin, the phenolic compounds content showed

Table 2. Effect of extraction solvent on total phenolic compounds contents at 60°Ca.

G.t.	Total Phenolics at 60°C			
Spices	Methanol	Ethanol	Acetone	LSD at p ≤ 0.05
Cloves	305.5 ^{c*}	548.0 ^b	781.0ª	53.2
Coriander	6.1 ^b	13.8 ^a	5.6 ^b	1.9
Cumin	20.8^{a}	20.8^{a}	14.8 ^a	8.7
Ginger	38.2ª	23.2 ^b	33.5 ^{ab}	11.0
Green Cardamom	23.1ª	8.4 ^b	4.5°	3.4
Turmeric	45.1°	82.9 ^b	164.4 ^a	13.4
Sumac	238.6°	317.4 ^b	725.1 ^a	68.6
Cinnamon	121.6°	214.5 ^b	293.4ª	18.7
Sweet Cumin	18.3 ^a	14.5 ^a	10.7ª	12.6
Sweet Laurel	29.0^{b}	47.3 ^a	9.1°	17.3

^aMeans are average of two replicates and expressed as mg of gallic acid/100g on dry weight basis. *Means with different letters in the same row are significantly different at $p \le 0.05$.

Table 3. Effect of extraction solvent on phenolic compounds contents at 40°Ca.

C	Total Phenolics at 40°C			
Spices —	Methanol	Ethanol	Acetone	LSD at p ≤ 0.05
Cloves	342.0 ^{a*}	493.4ª	445.2ª	159.0
Coriander	13.7ª	10.8^{ab}	7.2 ^b	5.0
Cumin	35.8 ^a	17.0 ^b	27.2 ^{ab}	16.9
Ginger	41.7ª	19.8 ^b	22.5 ^b	16.7
Green Cardamom	8.8 ^a	8.4^{a}	2.9ª	6.8
Turmeric	43.5°	77.2 ^b	123.6ª	21.0
Sumac	343.9 ^b	339.3 ^b	583.2ª	158.7
Cinnamon	182.1 ^b	160.0 ^b	315.1 ^a	61.2
Sweet Cumin	17.0ª	10.4 ^a	18.4ª	13.2
Sweet Laurel	$3.0^{\rm c}$	42.4ª	9.9^{b}	4.0

^aMeans are average of two replicates and expressed as mg of gallic acid/100g on dry weight basis. *Means with different letters in the same row are significantly different at $p \le 0.05$.

Table 4. Effect of extraction solvent on total phenolic compounds contents at ambient temperature^a.

C	Total Phenolics at 20°C			
Spices —	Methanol	Ethanol	Acetone	LSD at p ≤ 0.05
Cloves	394.7 ^{a*}	350.8 ^b	279.8°	27.0
Coriander	26.8ª	9.8^{b}	23.0^{a}	4.5
Cumin	43.8 ^a	19.0 ^b	26.9 ^b	15.7
Ginger	44.2ª	23.8 ^b	41.5 ^a	8.1
Green Cardamom	6.8 ^a	7.5 ^a	9.4ª	9.0
Turmeric	32.9 ^b	105.0 ^a	53.2 ^b	31.2
Sumac	271.4 ^b	342.8 ^b	754.5 ^a	89.4
Cinnamon	187.1 ^a	78.8 ^b	169.8 ^a	62.5
Sweet Cumin	25.5 ^a	10.3 ^b	11.3 ^b	5.0
Sweet Laurel	$0.9^{\rm c}$	52.9 ^a	18.3 ^b	9.5

^aMeans are average of two replicates and expressed as mg of gallic acid/100g on dry weight basis. *Means with different letters in the same row are significantly different at $p \le 0.05$.

similar values with different solvents. At 60°C, acetone proved to be anexcellent extractant for cloves, sumac, cinnamon and turmeric (781.0, 725.1, 193.4 and 164.4 mg GAE/100g respectively). While ethanol was excellent extractant for coriander and sweet laurel (13.8 and 47.3 mg GAE/100g respectively). But methanol was suitable extractant for cumin, ginger, green cardamom and sweet cumin (20.8, 38.2, 23.1 and 18.3 mg GAE/100g respectively).

As shown in **Table 3** the phenolic compounds content extracted at 40°C using methanol, ethanol and acetone, the total phenolic compounds content varied significantly among the extractants. Acetone found to be a superior extractant for phenolics from sumac, cloves, cinnamon and turmeric (583.2, 445.2, 315.1 and 123.6 mg GAE/100g respectively) at 40°C. Followed by methanol as extractant for coriander, cumin, ginger, and green cardamom; (13.7, 35.8, 41.7 and 8.8 mg GAE/100g respectively). Ethanol was suitable extractant for sweet laurel at 40°C (42.4 mg GAE/100g).

Table 4 shows the effect of different extractants (methanol, ethanol and acetone) on the total phenolic concentrations at the ambient temperature (20°C). The data depict that the three solvents significantly influenced the levels of phenolic compounds. When methanol was used as extractant at 20°C, the highest value of phenolic contentwas for cloves (394.7 mg GAE/100g), and the lowest value was for sweet laurel (0.9 mg GAE/100g). Cloves had the highest value of phenolic compounds when ethanol was used as extractant (350.8 mg GAE/100g), and the lowest value for phenolics was found in green cardamom (7.5 mg GAE/100g). On the other hand, sumac gave the highest concentration of phenolic compounds (754.5 mg GAE/100g) when acetone was the extractant, while green cardamom had the lowest concentration (9.4 mg GAE/100g) among the spices at 20°C.

Data proved that total phenolic compounds concentrations varied significantly according to extractant type and the temperature of extraction. This is in agreement with Hayouni *et al.* [14] and Hsu *et al.* [15] who reported that different extracting solvents influenced the total phenolic concentrations, which can be explained as a result of polarity of the solvent and thus different extractability among the phenolic compounds.

The level of total phenolic compounds for 36 vegetables and spices using 80% aqueous ethanol at room temperature, were studied [16], among them, turmeric, ginger, coriander, their values were higher than those obtained in this study, probably that was due to water ethanol mixture. Phenolic compound in spices like any other plant material, occur in groups or subgroups having different polarity which will influence the effectiveness of the solvent at a given temperature, and that explains why extraction of the same spice using the same solvent yields different level of phenolic compounds using different temperatures.

Shan *et al.* [17] studied the total phenolic compounds concentrations in cinnamon, coriander, cumin, sweet laurel and cloves, despite variation in extraction circumstances and the extraction temperature, our values are slightly higher than their values. The microbiological status and the chemical composition of the investigated spices were studied [18].

4.2. Effect of Extraction Temperature

Table 4 shows data on the concentrations of phenolic compounds extracted at 20°C, 40°C and 60°C using methanol as extractant. Cloves, ginger, turmeric, sumac, cinnamon and sweet laurel did not vary significantly by using different temperatures.

The highest concentrations of phenolic compounds were obtained by methanol at ambient temperature from cloves, sumac and cinnamon (394.7, 271.4 and 187.1 mg GAE/100g respectively). When the temperature was 40°C; the maximum extraction of phenolic compounds from sumac was 343.9 mg GAE/100g). However, 60°C was better for obtaining the extraction of phenolic compounds from green cardamom, turmeric and sweet laurel (23.1, 45.1 and 29.0 mg GAE/100g respectively). The highest phenolic compounds content in cloves, coriander, cumin, ginger, cinnamon and sweet cumin were detected at ambient temperature as compared with 40 and 60°C.

Table 2 shows the effect of temperature on the total phenolic compounds extracted by ethanol. The data show that there were no significant differences by different temperature especially for cumin, green cardamom, sumac, sweet cumin and sweet laurel. The rest of the spices varied significantly in total phenolic compounds using different temperatures. On the other hand, cloves, sumac and cinnamon showed significantly the highest content of phenolic compounds at 60°C (548.0, 317.4 and 214.5 mg GAE/100g respectively), followed by 40°C and at the ambient temperature, respectively.

The highest level of phenolic compounds were found in cloves, coriander, cumin, ginger, green cardamom, cinnamon and sweet laurel at 60°C as compared with 40 and 20°C as shown in **Table 4**.

Table 4 also shows the effect of temperatures (20, 40 and 60°C) on the total phenolic compounds content extracted by acetone. The data showed that there were significant differences in total phenolic compounds. At 60°C, cloves and turmeric had the highest content of phenolic compounds (781.0 and 164.4 mg GAE/100g respectively). At 40°C, cinnamon, cumin and sweet cumin had the highest levels of phenolics, values were 315.1, 27.2 and 18.4 mg GAE/100g respectively. Also, at 20°C; the total phenolics were for coriander, ginger, green cardamom, sumac and sweet laurel, values were 23.0, 41.5, 9.4, 754.5 and 18.3 mg GAE/100g respectively as shown in **Table 4**. Increasing the temperature from 20, 40 and up to 60°C will increase the total phenolic compounds level from cloves and turmeric and that is in agreement with data reported previously [18].

Wojdylo *et al.* [19] studied the levels of phenolics for cinnamon, cloves and turmeric; he reported values of 0.13 for the cinnamon, 8.96 for cloves and 1.72 mg GAE/100g for turmeric. These values are lower than values reported in the current investigation.

The type of extractant for phenolic had been regarded as a major issue for researchers. Suhaj [20] found that different spices reacted in different manner with respect to the extractant type, for instance; methanol was the most suitable extractant for cloves while the ethanol (96%) was best suitable for ginger. The cinnamon phenolics were extracted efficiently when 80% methanol with Soxhlet extraction were employed. The current investigations showed that acetone was a suitable extractant for cloves. On the other hand, ethanol was suitable to extract phenolics from ginger and that is in agreement with data reported previously [20].

Phenolic compounds reported for cloves, sumac and cinnamon found in agreement with similar values reported by Lin and Tang [21] on strawberry, green pepper and white onion.

4.3. Antioxidant Activity (Radical Scavenging Activity)

The scavenging of DPPH radicals was followed by monitoring the decrease in absorbance. IC_{50} (the efficient concentration of spice extract in mg/ml required to decrease initial DPPH radical concentration by 50%) was obtained by interpolation from linear regression analysis. The higher DPPH radical scavenging activity is associated with a lower IC_{50} values (good antioxidant activity). **Table 5** shows the antioxidant activity expressed as IC_{50} for the investigated spices. Antioxidant activity varied significantly among the spices, BHT was tested as reference synthetic antioxidant and showed the 50 % inhibition at 0.0193 mg/ml. For the investigated spices the lowest IC_{50} values were detected for cloves and cinnamon (0.077 and 0.09 mg/ml, respectively). On the other hand, coriander and green cardamom had the highest values of IC_{50} (1.051 and 1.26 mg/ml, respectively). The antioxidant activity expressed as IC_{50} for sumac, turmeric and sweet laurel did not vary significantly among the investigated spices (0.150, 0.160 and 0.170 mg/ml respectively), from the antiradical activity (1/ IC_{50}) in **Table 5**, the top five spices according to their antioxidant activity properties are: cloves, cinnamon, sumac, turmeric and cumin, all spices had lower antioxidant activity than the reference antioxidant BHT, these values are in agreement with values reported by Ereifej *et al.* [18].

Data on total phenolic compounds presented in **Table 4** suggest that the type of phenolic compounds rather than the amount is responsible for antioxidant activities [5] [22].

Al-Ismail *et al.* [23] studied the antioxidant activities for a set of plant extracts such as cumin, sumac, sweet cumin, sweet laurel and others; these extracts were obtained using the water and the ethanol extractants. Although the amount of phenolic compounds was high in sumac and cumin, these spices had low levels of antioxidant activities, which were attributed to the differences in type of phenolic compounds in each spice. Generally, it is suggested that it is difficult to compare between the antioxidant activities of spices without considering

Table 5. DPPH radical scavenging (IC₅₀) and antiradical activity values for the spices marketed in Jordan^a.

Spices	IC ₅₀ (mg/ml) ^b	1/IC ₅₀ (Antiradical activity)
Cloves	0.077^{g}	12.99 ^b
Coriander	1.051 ^b	0.95^{d}
Cumin	$0.520^{\rm e}$	1.92 ^d
Ginger	$0.600^{\rm d}$	1.67 ^d
Green ardamom	1.260^{a}	0.79^{d}
Turmeric	0.160^{f}	6.25 ^{bcd}
Sumac	$0.150^{\rm f}$	6.67 ^{bcd}
Cinnamon	$0.090^{\rm g}$	11.11 ^{bc}
Sweet Cumin	0.650°	1.54 ^d
Sweet Laurel	0.170^{f}	5.88 ^{cd}
ВНТ	$0.0193^{\rm h}$	51.81 ^a
$LSDP \leq 0.05$	0.041	6.98

^aMeans are average of two replicates and expressed as mg/ml. * Means followed by different letters in the same column are significantly at P ≤ 0.05; b IC₅₀ is the efficient concentration of the test spices that scavenge (decrease) 50% of stable DPPH radical.

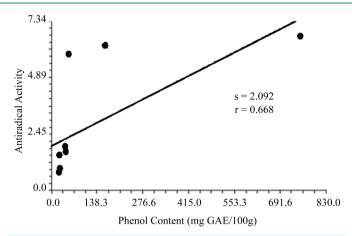


Figure 1. Relationship between total phenolic compounds content and the antiradical activity for the spices.

the extractant and extraction method as well as the types of phenolic compounds in spices.

Kaur and Kapoor [16] studied the antioxidant activity for ginger, coriander and turmeric. He found that the antioxidant activity ranged from 60 to 80%. The current study reports that there was a relationship between the antioxidant activity and the total phenolic compounds content (**Figure 1**).

The antioxidant activity for cloves, sweet laurel, coriander, cinnamon and sweet cumin using percent of inhibition for DPPH approach was studied [24]. Our observations found to be in agreement with that reported by Politeo *et al.* [24].

Figure 1 shows the correlation between the antiradical activity and total phenolic compounds of the investigated spices, antiradical activity is $1/IC_{50}$ and directly proportional to the antioxidant activity. Positive correlation (r = 0.67) was found between antiradical activity and total phenolic compound levels.

5. Conclusion

In conclusion the level of phenolic compounds extracted by methanol, ethanol and acetone at 20°C, 40°C, and 60°C varied significantly among the spices. The highest level of phenolics was obtained with methanol at 60°C. The level of phenolics was increased when temperature was elevated from 20 to 40 and 60°C. Cloves had the highest antioxidant activity whereas green cardamom and coriander had the lowest values. Antioxidant activity found to be positively correlated with total phenolic compounds.

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