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Quantitative Estimation of Total Polyphenol Content and Antioxidant Capacity of the four variety of Spices cultivated in Ethiopia

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Abstract

Natural products such as herbs, fruits, and spices, are becoming more popular among the scientific community and consumers because of their potential to arrest the effects of free radicals in the human system. Mainly this study focuses on due to consumers concern about health effects it is believed to study phenolic content and its antioxidant activities of fenugreek, cardamom, turmeric and coriander. Among studied species samples the total phenolic content of methanol and acetone extracts of turmeric rhizome was the highest at 11.420±0.022 and 10.994±0.121 mg/g followed by ethanol and acetone extracts of fenugreek extracts 5.357±0.192 and 4.785±0.201mg/g respectively, while the lowest polyphenol content was determined in cardamom 1.047±0.112 mg/g. The results of this study revealed that methanol and acetone extracts of cardamom had the highest antioxidant activity of 90.615±0.213% and 90.213±0.111% followed by acetone extracts of coriander 88.589±0.081% and methanol extracts of fenugreek 88.241±0.124% respectively. The lowest antioxidant capacity was estimated using the ethanol extracts of coriander 32.901±0.177%. According to the present study high consumption of cardamom, fenugreek, coriander and turmeric as a spices and medicinal purpose may be used as special source of antioxidants for free radical scavenging.

Keywords: Polyphenol; Antioxidant; Free Radical; Spices; IC_{50}; DPPH

List of abbreviation: RA: Regional Anesthesia; SA: Spinal Anesthesia; C/S: Cesarean Section; WHO: world Health Organization; DURH: Dilla university referral hospital; TVP: Trans-vesicalprostectomy; PROP: Pre operation

Introduction

Plants are potential sources of natural bioactive compounds such as secondary metabolites and antioxidants. They absorb sun light and produce high levels of oxygen and secondary metabolites via photosynthesis [1]. As antioxidants, polyphenols from plants can rule out the deleterious effects of oxidation through many ways including chelating metal ions, preventing the accumulation of ROS, empowering antioxidant enzymes, and inhibiting lipid peroxidation [2]. Ethiopia mainly produces ginger, turmeric, cumin, rosemary, cardamom, capsicum, fenugreek, coriander, korarima, Timiz, black pepper, hot pepper, rue, celery and thyme [3]. Plant secondary metabolites are sources for numerous natural products such as spices, vegetables, herbs, fruits. This is becoming more popular in the world of research because of their ability to detoxify free radicals from the biological system [4]. Interest in bioactive phenolic compounds in plant-based products is currently growing. Recent research has shown culinary herbs as a dietary source of antioxidant polyphenols which has increased interest in the study of their phenolic composition and antioxidant properties [5].

The use of herbal plants as natural antioxidant and antimicrobial agents has caught some interests, considering that there are growing consideration about side effect of using synthetic compounds and increasing bacterial resistant toward antimicrobial agents [6].

The most important bioactive constituents of medicinal plants are the alkaloids, tannins, flavonoids, and phenolic compounds [7]. Spices and herbs are excellent sources of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids which have been reported to show good antioxidant activity [8]. Plant phenolics have potential health benefits mainly due to their antioxidant properties such as reactive oxygen species (ROS) scavenging and inhibition, electrophile scavenging and metal chelation [9]. The Pharmacological activities of coriander seeds have been connected to their chemical profile in different preparations, with coriander essential oil and various extracts expressing antimicrobial, anticarcinogenic, antioxidant and antidiabetic activities [10]. Fenugreek is used in functional foods, traditional foods, and nutraceuticals as well as in physiological applications such as antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant, and antidiabetic agents [11].

Turmeric has been used as a dye and spice. Turmeric is an important tropical spice mainly due to its color, aroma and antioxidant properties. The yellow color in turmeric is mainly due to the presence of 3 major pigments; curcumin 1, 7-bis (4-hydroxy-3-methoxyfenil)-1, 6-heptadiene-3,5dione), demethoxy-curcumin and bis demethoxycurcumin [12]. Cardamom seeds, pods, leaves, rhizomes and flowers are used in traditional medicine and as spices in southern Ethiopia [13]. Considering that the main objective of this study was to determine the level of total polyphenol in the selected fenugreek, coriander, turmeric, and cardamom and to evaluate these species as new potential sources of natural antioxidants. In this study, the extracts of all samples were prepared using methanol, ethanol, and acetone, because organic solvents have different polarities and therefore have different natures to extract the compounds.

Material and Methods

Chemicals and reagents

The following chemicals and reagents were used to determine polyphenol content and antioxidant potential of all analyzed spices. DPPH, Folin-Ciocalteu phenol reagent, sodium carbonate, gallic acid, methanol, ethanol, acetone, nitric acid, ascorbic acid, and hydrochloric acid were used. All chemicals and reagents used were of analytical grade.

Sample collection and preparation

The seeds of Coriander, fenugreek, cardamom, and turmeric rhizome samples were collected from the local market of Arba Minch Ethiopia. After sampling, all species samples were processed for the complete removal of moisture content. The Coriander, fenugreek, cardamom, seeds and turmeric rhizomes were carefully washed under tap water followed by, distilled water and shade dried for 7 days. The dried samples were ground to a powder and stored in airtight containers.

Extraction

Spices secondary metabolites possess various biologically active components and highly polar organic compounds such as methanol, ethanol, and acetone were used for extraction because they are capable of extracting a wide range of polar compounds. Each of the five powdered samples (5g) was soaked in a conical flask containing 100 mL of methanol 90 % (9:1), ethanol 70 % (7:3), and acetone 80% (4:1) for 24 h. The Conical

flask was allowed to stand for 20 min in a water bath (at 100°C) with occasional shaking followed by keeping all the flasks on an electrical shaker at 200 rpm for 24h. Each extracted sample was filtered through wrapped muslin cotton close followed by; sterilized Whatman No. 1 filter paper and finally the supernatant were stored at 4°C in a refrigerator for further use.

Determination of Total phenolic content (TPC)

The total phenolic content of each sample was estimated using the Folin–Ciocalteu method described by Tanzeela et al [14] with slight modifications. Folin-ciocalteu reagent was prepared by diluting with 1mL of Folin-ciocalteu with 10 mL distilled water at a ratio of v/v (1:10). Briefly 0.25 mL of each sample extract mixed with 1.25 mL of Folin-ciocalteu reagent. After 5 min of incubation, 1 mL of 7.5 % Sodium carbonate was added to the mixture and mixed homogenously using a vortex mixer and diluted with distilled water to a final volume of 5 mL. After one hour incubation the absorbance of each solution was measured at 760 nm using a double beam UV-visible spectrophotometer. Gallic acid was used to plot the calibration curve using different concentrations (6-150 mg/L) to determine the total polyphenol content of each sample extract. Total phenolic content was expressed as milligrams of gallic acid equivalent per gram (mg GAE/g DW) of the extract by calculating using a standard calibration curve. All values were estimated in triplicates using the following formula:

$$P = \frac{C.V}{m} \quad (1)$$

where P = is the total phenolic content (mg/g plant extract, in GAE) c = Concentration of gallic acid (mg/mL), V = Volume of extract (mL), and m = Weight of pure plant extract (g).

Ethiopia produces different types of spices and is used for fragrance, color, taste and smell as a prepared dish. Due to consumers concern about health benefits, the following spices were studied.

Table 1: Type and parts used for phenolic and antioxidant analysis

Type of spices	Parts used	Local Name	Scientific name	Botanical family
Turmeric	rhizome	Irid	Curcuma longa	Zingiberaceae
Fenugreek	seed	Abish	Trigonella foenum graecum	Fabaceae
Coriander	seed	Dimbilal	Coriandrum sativum L.	Apiaceae
Cardamom	seed	Korarima	Elettaria cardamomum	Zingiberaceae

Determination of Antioxidant Capacity

The antioxidant capacities of all sample extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical scavenging method [9]. Free radical Scavenging of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) represents the free radical reducing activity of extracts based on electron reduction. DPPH is a stable free radical that can be scavenge by the sample components and it is taken as the reference for testing whether the sample medicinally active or not. The reaction mixture contained 2 mL of 0.003% of DPPH with l mL of each sample extract of varying concentrations (0.1-1.2 mg/ml) in methanol. The decreasing absorbance of the reaction mixture were measured using double beam UV-vis spectrophotometer at 517 nm against the blank solution which containing solvents and reagents in place of sample extract. The ascorbic acid was used as a positive control because it is highly purified compound with the highest DPPH radical scavenging abilities. Evaluation of DPPH free radical scavenging abilities were carried out in triplicates for each sample components and values were reported as mean of those triplicate results. The percent inhibition of sample extract using DPPH radical was calculated by the equation:

$$\text{Inhibition}(\%) = A_{DPPH} - A_{sample} / A_{DPPH} x100 \quad (2)$$

Where, A_{DPPH} is the absorbance of control and A_{sample} is the absorbance of reaction mixture

Statistical analysis

The obtained data were expressed as mean ± standard error of the mean. After determining and analysis of all obtained data, the result of this study will be qualitatively and quantitatively determined using MS-Excel and origin software version six for the calibration curves of polyphenol content, antioxidant potential of the result in tems of extracting solvent.

Results and Discussion

Determination of Phenolic content

Total phenolic contents of selected spices extract were evaluated using Folin-Ciocalteu reagent as oxidizing agent. The mean phenolic content values of all selected spices samples were shown Table 2 below. The total polyphenol content determined from spices samples were ranged from 1.047 ± 0.112 to 11.420 ± 0.022 mg GAE/g. The comparative study result Table 2 clearly depicts that methanol and acetone extracts of turmeric had the highest total phenolic contents 11.420 ± 0.022 mg GAE /g and 10.994 ± 0.121mg GAE/g followed by ethanol and acetone extracts of fenugreek 5.357 ± 0.192 mg GAE/g and 4.785 ± 0.201mg GAE/g respectively. For methanol and ethanol extracts of turmeric, calculated value of this study lower than reported by Array et al [15] with the calculated value of 15.71 and 40.80 mg GAE/g respectively. The lowest phenolic content was estimated in ethanol extract of Cardamom 1.047 ± 0.112 mg GAE/g.

In case of coriander using different solvent extracts, the highest phenolic content was recorded using ethanol extracts 4.782 ± 0.211 mg GAE/g followed by methanol extract 3.136 ± 0.073 mg GAE/g. For this study methanol extract of coriander was in agreement with Sema et al [16] but higher than ethanolic extract with estimated values 4.2±0.3 and 2.1±0.4 mg GAE/g respectively. The lowest concentration of phenolics was estimated 2.589 ± 0.091 mg GAE/g using acetone extracts of coriander but the values strictly greater than the result of Paolino et al [17] who recoreded 1.342 mg/g. Briefly acetone and ethanol extracts of fenugreek were also in agreement with Syeda et al [18] with estimated values 4.04±0.004 and 6.85 ± 0.002 mg GAE/g respectively, but methanol extract 5.75±0.002 mg GAE/g was higher than this study. As the result of cardamom in Table 2 showed that the highest value of phenolic content was obtained 2.228 ± 0.091mg/GAE/g using methanol extract followed by acetone extract 1.908 ± 0.061 mg GAE/g and the lowest phenolic values was recorded in ethanol extract 1.047 ± 0.112 mg GAE/g.

Even though the value of phenolic concentration in turmeric was very high in both methanol and acetone extracts its concentration using ethanol was comparatively lower 3.134 ± 0.051mg GAE/g. As the result of the analysis indicates phenolic contents were the highest in methanol extracts from all samples except fenugreek samples. Previous study reported that the variability in the total phenolic contents in different extracts could be the result of many reasons like: varying solubility of the phenolic compounds, method of extraction, the region of the plant, the variation in solubility may be driven by the solvent polarity [19]. The higher level of polyphenol content in all samples estimated using polar organic solvent indicates that the polar components of phenolic compounds highly extracted by polar solvents. Using plant origion the decreasing order of polyphenol content in the selected spices noticed as: turmeric >fenugreek > Coriander > cardamom. The health benefits of phenolics are primarily derived from their antioxidant potentials because the radicals produced after hydrogen or electron donations are resonance stabilized and thus relatively stable. To counter the potential hazards of oxidative damage, the dietary consumption of antioxidant phenolics including phenolic acids and flavonoids may be regarded as the first line of defense against highly reactive toxicants [20]. All measurements were made in triplicates

Table 2: Total Phenolic content of each sample in mean ± Standard deviation

Sample type	Total Polyphenol content (mg/g) in different Solvents extracts		
	Methanol	Ethanol	Acetone
Turmeric	11.420 ± 0.022	3.134 ± 0.051	10.994 ± 0.121
Coriander	3.136 ± 0.073	4.782 ± 0.211	2.589 ± 0.091
Fenugreek	3 .058 ± 0.139	5.357 ± 0.192	4.785 ± 0.201
Cardamom	2.228 ± 0.091	1.047 ± 0.112	1.908 ± 0.061

Secondary metabolites produced in plants are low molecular weight natural products. phenolic compounds are widely distributed in plants that have been reported to exert multiple biological effects including antioxidant, free radical scavenging, antiinflammatory, and anticarcinogenic [21]. Among all samples analyzed to determine its phenolic content turmeric provides the greater number of hydrogen atoms to neutralize the number of free radicals on oxidative stress.

Antioxidant activity of spices

A large number of plants have been screened as a viable source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds which are responsible for maintenance of health and protection from different diseases [22]. For DPPH radical scavenging antioxidant activity analysis, ascorbic acid was used as a standard, because it contains relatively high positive values correlated with both antioxidant activity and total phenolic content. The antioxidant capacities of different extracts of selected spices were evaluated using different polarities of organic solvents and the result is relatively positive. As the results of the study Table 3 showed that all solvent extracts exhibited the best antioxidant activity regarding the sample origion. This is attributed by ethanol, methanol and acetone having the power to extract more phenolic antioxidants.

The result of this study Table 3 revealed that methanol and acetone extracts of cardamom have the highest antioxidant activity 90.615± 0.213% and 90.213±0.111% followed by acetone extracts of coriander 88.589±0.081% and methanol extracts of fenugreek 88.241± 0.124% respectively. The lowest antioxidant capacity was estimated using ethanol extracts of coriander 32.901±0.177%. Comparative analysis from Table 3 and figure 1 depicts that methanol extract of turmeric showed the highest antioxidant capacity 79.030 ± 0.085% followed by ethanol extract 74.820±0.172%. This values agreed with reports of Array et al [15] who obtained maximum of methanol and ethanol extracts of turmeric 85% and 90% respectively. But the result significantly higher than Sepahpour et al [23] who determined methanol and ethanol extracts of turmeric 27.8% and 47.4% respectively. The lowest antioxidant capacity was recoreded using acetone extracts 58.679±0.063, this result strongly agreed for Sepahpour et al [23] who estimate values 67.8%. For this study, methanol and acetone regarded as the best solvents to be used for a maximal extraction of phenolic compounds for all selected spices with good antioxidant activity.

It has been demonstrated that phenolic antioxidants have the ability to donate their hydrogen atoms for the stabilization of free radicals generated by the oxidative stress or to reduce and chelate transition metals that facilitate the generation of these radicals [15].

Table 3: Maximum antioxidant activity for each sample using different solvent extracts

Sample type	Inhibition (%) using different solvent extract		
	Methanol	Ethanol	Acetone
Turmeric	79.030 ±0.085	74.820±0.172	58.679±0.063
Coriander	87.914±0.049	32.901±0.177	88.589±0.081
Cardamom	90.615± 0.213	47.791±0.084	90.213±0.111
Fenugreek	88.241± 0.124	46.986±0.126	85.421± 0.234

All measurements were made in triplicates and reported mean ± SD

According to the findings of this study, the DPPH radical scavenging activity of cardamom has greater antioxidant capacity than the turmeric, coriander, and fenugreek whereas turmeric was slightly lower among all others. The antioxidant activities of methanol and acetone extracts of coriander from Table 3 and Figure 2, 87.914±0.049 % and 88.589±0.081% support the activity shown by methanol extract 88.241± 0.124% and acetone extract of fenugreek 85.421± 0.234%. The results from Table 3 showed that all solvents give good positive results for antioxidants of selected spices. Based on the result of the analysis Table 3 and different figures below, the decreasing order of antioxidants obtained as cardamom > coriander > fenugreek > turmeric. Comparatively the mean values of the decreasing order between solvent extracts were as follows; methanol > acetone > ethanol. The antioxidant activity of spices can be influenced by many factors. The most significant are those related to the botan-

ical origin and antioxidant content of the spices. However, the literature suggests that the amount of antioxidants in the sample also depends on the type of solvent used for their extraction [24]. For this study solvents with different polarities methanol, ethanol and acetone have been used for estimation of antioxidant capacities. Those solvents give different results with its variety of spices samples. The use of antioxidants in lipid-containing foods is one method used to minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase the shelf life of food products [25].

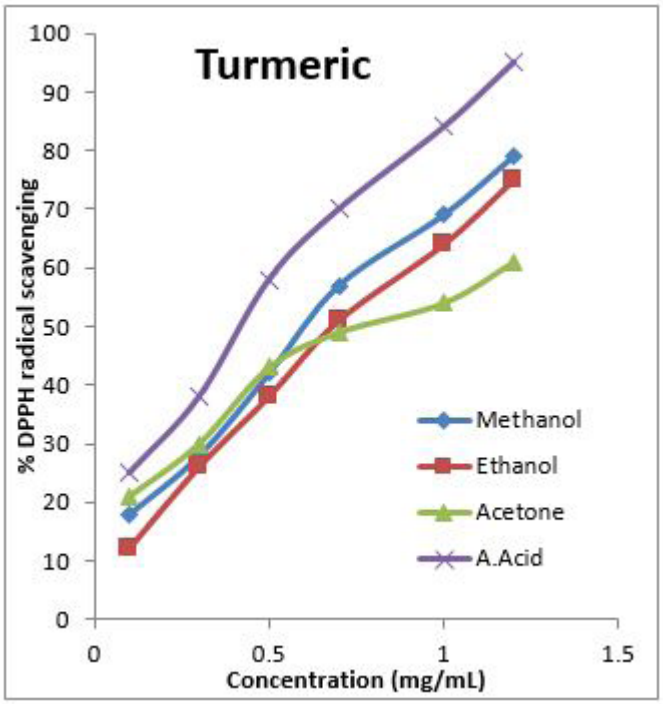

Figure 1: inhibition (%) of turmeric

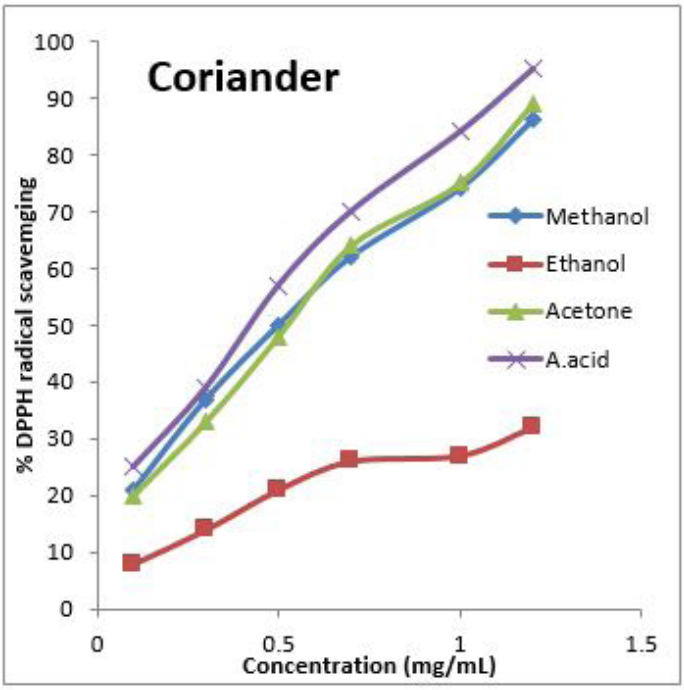

Figure 2: inhibition (%) of coriander

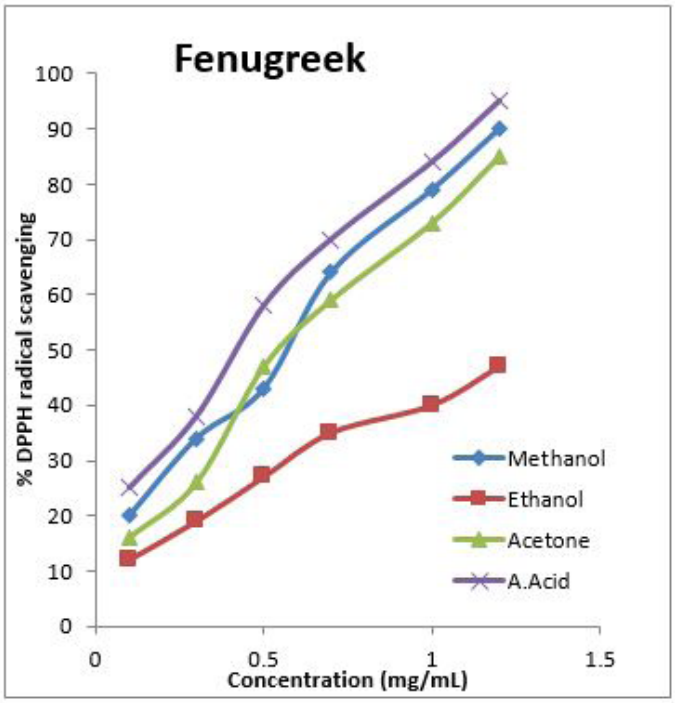

Figure 3: Inhibition (%) of fenugreek

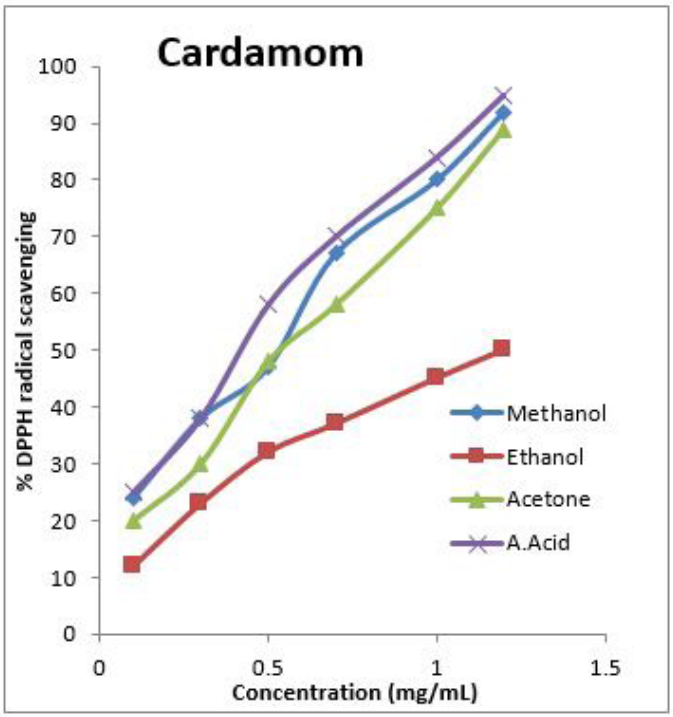

Figure 4: inhibition (%) of cardamom

Determination of IC_{50} values

The IC_{50} value is the concentration of a substrate that causes 50 % loss of the DPPH activity and was calculated by linear regression of plots of the percentage antiradical activity against the concentration of the tested compounds [26]. The IC_{50} values of all sample extracts using different solvents were estimated and the results were given in Table 4.The lower the IC_{50} value, the higher is DPPH free radical scavenging capacity of all spices extracts. The cardamom methanol extract exhibited the lowest IC_{50} value, i.e., 0.506 ±0.194 when it was compared with other sample components with their respective extracting solvent. Based on the IC_{50} values of the calculated samples, methanol extracts of coriander and fenugreek showed similar IC_{50} values 0.545 ±0.172, and 0.556±0.210 mg/mL respectively. The IC_{50} values of turmeric methanol extracts gives the 0.654±0.235 mg/mL. Comparatively coriander ethanol extracts showed the highest IC_{50} values 2.021±0.520 mg/mL followed by fenugreek1.273 ±1.320 mg/mL and the lowest value was observed in turmeric 0.734±1.012 mg/mL with the same solvent. The largest IC_{50} values of turmeric using acetone extract was 0.832± 0.127 mg/mL followed by fenugreek 0.618±0.403 mg/mL and the lowest value was recoreded in coriander 0.555± 1.302 mg/mL. All sample extracts revealed that the IC_{50} values using different solvents were above the IC_{50} values of ascorbic acid using methanol, ethanol and acetone extracts respectively 0.445 ±0.125, 0.447±0.941, and 0.446±0.372.

Table 4: IC50 value of DPPH radical scavenging ability of extracts

Sample type	IC_{50} values (mg/mL) of DPPH radical scavenging		
	Methanol	Ethanol	Acetone
Turmeric	0.654±0.235	0.734±1.012	0.832± 0.127
Coriander	0.545±0.172	2.021±0.520	0.555± 1.302
Cardamom	0.506±0.194	1.140±0.182	0.580±1.540
Fenugreek	0.556±0.210	1.273 ±1.320	0.618±0.403
Ascorbic acid	0.445±0.125	0.447±0.941	0.446±0.372

Conclusion

As the findings of this study showed that the methanol and acetone extracts of turmeric had the highest phenolic contents as antioxidant activities. But, ethanolic extract was the lowest relative to the two other solvents. Among selected spices extracts, polyphenolic content in turmeric was the highest followed by fenugreek and the lowest polyphenol content estimated in cardamom. Briefly Coriander showed moderate polyphenol contenet. Methanol is the best solvent for the extraction of phenolic antioxidant compared to ethanol and acetone. Significantly antioxidant activity of cardamom extract was higher than that of all other studied spices. Methanol and acetone extracts of cardamom was the highest in antioxidant activities which could inhibit the distribution of free radicals better as compared to methanol, acetone and ethanol extracts of turmeric, coriander, fenugreek samples. Generally it can be concluded that selected spices samples cardamom, fenugreek, coriander and turmeric were good source of phenolic antioxidants and they can reduced the harmful effects produced by free radicals.

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Conflict of interest

Authors declared that they have no conflict of interest

Financial disclosure

The authors declared with no financial interest

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