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Research article

In vitro antioxidant activity and polyphenolic content of commonly used spices from Ethiopia

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ABSTRACT

Introduction: In this study, the antioxidative effectiveness, and polyphenolic content of methanol and aqueous extracts of spices such as *Lippia adoensis* (Koseret), *Nigella sativa* (Thikur azmud), *Piper capense* (Timiz), *Thymus schimperi* (Tosign) and *Trachyspermum ammi* (Netchazmud), consumed among people of Ethiopia were investigated.

Methods: The antioxidant activity was assessed *via* established *in vitro* assay models such as 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical quenching assay, reducing power assay and reactive nitrogen species (RNS) inhibitory potential. Total phenolics content was measured according to Folin-Ciocalteu's method and total flavonoid content was estimated by using Aluminium chloride colorimetric method.

Results: The results showed that the total phenolic content was highest in both methanol (720 ± 0.04 mg GAE/100 g extract DW) and aqueous (580 ± 0.08 mg GAE/100 g DW) extracts of *L. adoensis*. Among the five tested spices, the methanol and aqueous extracts of *L. adoensis* exerted the strongest DPPH radical quenching activity with IC_{50}values of 49.17 ± 1.26 and 20.99 ± 8.6 μg/mL, respectively. Both methanol and aqueous extracts of *L. adoensis* showed notable reducing capacity. The highest RNS scavenging activity was shown by both methanol (IC_{50} 597.21 ± 6.99 μg/mL) and aqueous (IC_{50} = 551.5 ± 28.9 μg/mL) extracts of *L. adoensis*. High to moderate positive correlations were observed between total phenolic contents and *in vitro* antioxidant assays. This indicates that the antioxidant activities of the tested spices are attributed to the phenolic contents.

Conclusion: The results of the present work revealed that the tested spices demonstrated high phenolic contents and antioxidant properties. Thus, these spices are worth considering as important sources of natural antioxidant agents.

1. Introduction

Spices are part of the daily food in several parts of the world. They are being used as food additives mostly for their organoleptic attributes (Tapsell et al., 2006). It is understood that spices also exhibit effects that are beneficial to the health of humans apart from enhancing the taste and flavor of food (Chandrasekhara and Srinivasan, 1999; Słowianek and Leszczyńska, 2016). Consumption of spices has been reported to help in the prevention of cardiovascular diseases, carcinogenesis, inflammation, atherosclerosis, etc. (Srinivasan, 2005; Kaefer and Milner, 2008; Jungbauer and Medjakovic, 2012; Mohammed and Islam, 2018). Spices are abundant sources of compounds, which have strong antioxidant properties to serve as substitute for synthetic antioxidants in food systems to get additional health benefits. They are known to be rich in antioxidant phenolic compounds (Cai et al., 2004; Kim et al., 2011; Hossain et al., 2008; Loizzo et al., 2010; Viuda-Martos et al., 2011). The antioxidant properties of spices have been evaluated by several researchers (e.g., Kahkonen et al., 1999; Zheng and Wang, 2001; Gulcin et al., 2004; Satyanarayana et al., 2004; Shan et al., 2005; Wojdyło et al., 2007; Bouba et al., 2010; Dada et al., 2013; Nagy et al., 2015; Słowianek and Leszczyńska, 2016; Hashem et al., 2018).

Because of its diverse physiographic and climatic conditions, Ethiopia is endowed with enormous plant diversity of which some, especially spices, are used for food and/or medicinal value. In Ethiopia, spices are either cultivated or collected from the wild for daily use as food or remedy for human ailments. In Ethiopia, Koseret (*Lippia adoensis* [Otto & A. Dietr] Cufod. Verbenaceae), Tikurazmud (*Nigella sativa* L., Ranunculaceae), Timiz (*Piper capense* L.f., Piperaceae), Tosign (*Thymus schimperi* L., Lamiaceae) and Netchazmud (*Trachyspermum ammi* (L.) Sprague ex Turrill, Apiaceae) are the well-known spices used for the preparation of

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several foods and beverages. Several ethnobotanical studies (e.g., Asfaw et al., 2000; Megersa et al., 2013; Kefalew et al., 2015; Esubalew et al., 2017; Jima and Megersa, 2018; Tefera and Kim, 2019) conducted in Ethiopia showed that these plants are also used in traditional medicine in different parts of the country. Aqueous and ethanol extracts from leaves of *L. adoensis* were reported to possess analgesic activity (Makonnen et al., 2003) and anti-pyretic activity (Debell et al., 2005). The antioxidant properties in tandem with terpenoid contents of the essential oil of some of these spices have been reported by some researchers (e.g., Sishu et al., 2005; Eyob et al., 2008; Menghesa et al., 2011; Hailemariam and Shimelis, 2013; Bizuayehu et al., 2016; Debebe et al., 2018; Simur, 2018). However, antioxidant properties of these spices were not comparatively investigated from a standpoint of their phenolic contents. Thus, the aim of this study was to comparatively evaluate the phenolic contents of aqueous and methanolic extracts of these spices and correlate with their antioxidant potentials.

2. Materials and methods

2.1. Chemicals

All the chemicals and solvents used were of analytical grade. 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH), potassium ferricyanide, ferric chloride, butylated hydroxytoluene (BHT), L-ascorbic acid, quercetin, catechin, gallic acid, sodium nitroprusside, N-(1-naphthyl) ethylenediamine dihydrochloride and TCA were purchased from Merck Co. India. Glass distilled water was used to prepare all reagents and solutions.

2.2. Spices collection

For phytochemical and antioxidant activity analyses, we used leaves of *L. adoensis* and *T. schimperi* as these parts are used in food and/or traditional medicine preparations. Fresh healthy leaves of *L. adoensis* (from three different randomly selected mature wild grown plants) were collected in separate polyethylene bags from around Haramaya University, eastern Ethiopia. Similarly, fresh leaves of *T. schimperi* were collected from the same area from plants grown in individual's garden. On the other hand, seeds collected from three randomly selected local spice seed vending shops (in Harar city) were used for the same purpose in the case of *N. sativa*, *P. capense* and *T. ammi* as seeds are the parts consumed by local Ethiopians. The plant specimens were identified by comparing with preserved specimens and authenticated by botanist at Haramaya University Herbarium.

2.3. Extract preparation

The plant samples (leaves and seeds) were first separately washed using tap water, rinsed with distilled water and dried in an oven (Bluefic, India) (at 40 °C, 96 h) to a constant weight. The dried samples were then pulverized using mortar and pestle. The powder of each spice (50 g) was extracted (in triplicate) by separately dissolving in 250 ml of methanol and distilled water for 24 h. After 24 h of soaking, the extract was filtered using Whatman no.1 filter paper and the filtered solvent was evaporated under reduced pressure using a rotary evaporator (Heidolph rotary evaporator, Laborata 4001) at 55 °C to remove the solvent and the dried extract was then stored in the refrigerator (at 4 °C) until analysis.

2.4. Determination of total phenolic content

The total phenolics content in methanol and aqueous extracts of the spices were determined according to the Folin–Ciocalteu procedure (Singleton et al., 1999). About 4 mg of the dried crude extract was mixed with 5 ml 80% acetone, shaken well in a vortex-shaker and centrifuged at 2,200 × *g* for 2 min at room temperature. The supernatant was transferred to a 10 mL volumetric flask with a Pasteur pipette. The remaining residue was extracted twice with 2.5 mL 80% acetone, shaken well in a vortex-shaker, centrifuged as before after standing for 5 min and the supernatants were transferred to the same 10 mL volumetric flask with a Pasteur pipette. Aliquots (100 μL) of supernatant from each sample were put into a 10 mL volumetric flask and mixed with 1.9 mL deionized water. Folin–Ciocalteu–phenol reagent (1 mL) was added and the solution was shaken vigorously and mixed with 5 mL sodium carbonate (20%). After 20 min, the solution was centrifuged at 2,200 × *g* for 2 min at room temperature. Absorbance at 735 nm was measured in a spectrophotometer (Shimadzu UV-2401PC) and the results were expressed as gallic acid equivalents from a gallic acid standard curve (mg GAE/100 g^{-1} Extract DW; $r^2 = 0.9867$). The analyses were performed in triplicate.

2.5. Determination of total flavonoid content

The total flavonoid content in the methanol and aqueous extracts of the spices was determined by aluminum chloride colorimetric method based on the method indicated by Ordonez et al. (2006). Briefly, a volume of 0.5 mL of $AlCl_3$ethanol solution (2%) was added to 0.5 mL of extract. After one hour incubation at room temperature, the absorbance was measured at 420 nm using UV-Vis spectrophotometer. The analysis was performed in triplicate. The total flavonoid content was estimated from a quercetin standard curve and the results are expressed as mg quercetin equivalents (mg QE/100 g^{-1} Extract DW; $r^2 = 0.9675$).

2.6. DPPH radical quenching capacity

The determination of DPPH stable radical scavenging activities of the extracts and standards were evaluated based on the method described by Singh et al. (2002). Extracts (1mL) of the different concentrations (i.e., 10–1000 μg/mL^{-1}) made by reconstituting in respective solvents were added to DPPH solution (5 mL, 0.1 mM) in methanol and vortexed. After 20 min of reaction at 25 °C, the absorbance was measured at 517 nm against a blank (methanol) in a UV-Vis spectrophotometer (Shimadzu UV-2401PC). Methanolic DPPH solution (5 ml) without antioxidant was used as control. The DPPH scavenging activity of the extract was expressed as IC_{50} (inhibitory concentration), that is, the concentration of the extract at which DPPH radicals were quenched by 50%. Ascorbic acid and Butyl Hydroxy Toluene (BHT) were used as standard antioxidants.

The percentage quenching of DPPH was calculated as follows:

$$\text{DPPH} \bullet \text{quenching capacity}(\%) = \frac{(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

Where, $\text{Abs}_{\text{sample}}$ is absorbance of the sample (extract and standard antioxidant) and $\text{Abs}_{\text{control}}$ is DPPH solution without the added extract.

2.7. Ferric reducing antioxidant capacity

The reducing powers of the extracts and the positive standard controls were determined by using the potassium ferricyanide reduction method (Oyaizu, 1986). Extracts (0.5 mL) of the different concentrations (i.e., 10–1000 μg/mL^{-1}) that were made by reconstituting in respective solvents were mixed with 2.5 mL of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) solution and vortexed. After incubation at 50 °C in oven for 20 min, 2.5 mL of Trichloroacetic acid (TCA) (10%, w/v) was added to all the tubes and centrifuged at 3,000 x *g* for 10 min. Afterwards, upper layer of the solution (or supernatant) (5 mL) was taken and mixed with deionized water (5 mL). To this solution, 1mL of $FeCl_3$ (1%) was added in each test tube and incubated at 35 °C for 10 min. The formation of Perls Prussian color was measured at 700 nm in a UV-Vis spectrophotometer (Shimadzu UV-2401PC). Increased absorbance of the reaction mixture indicates increased reducing power. Ascorbic acid and Butyl Hydroxy Toluene (BHT) were used as standard antioxidants.

2.8. The Reactive Nitrogen Species (RNS) inhibition capacity

Nitric oxide (NO) generated from sodium nitroprusside (SNP) in aqueous solution at physiological pH was estimated by the use of Griess reaction (Green et al., 1982). The reaction mixture (3 mL) containing SNP (10 mM, 2 mL), phosphate buffer saline (0.5 mL, pH 7.4) and the extracts (0.5mL) at different concentrations (10–1000 μg mL^{-1}) were incubated at 25 °C for 150min. After incubation, 0.5 mL of the incubated solution containing nitrite was pipetted and mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of N-(1-naphthyl) ethylenediamine dihydrochloride was added, mixed and allowed to stand at 25 °C for 30 min. The absorbance of pink colored chromophore formed during diazotization was measured at 540 nm. Butyl Hydroxy Toluene (BHT) and catechin were used for comparison. The NO scavenging activity of the extract was expressed as IC_{50} (inhibitory concentration), that is, the concentration of the extract at which NO radicals were quenched by 50%.

$$\text{Nitric oxide scavenging capacity}(\%) = \frac{(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100 \quad (2)$$

Where, $\text{Abs}_{\text{sample}}$ is absorbance of the sample and $\text{Abs}_{\text{control}}$ is absorbance of control.

2.9. Statistical analysis of data

Data obtained were analyzed using General linear model to check the impact of spice type, extraction solvent and extract concentrations as main effects and their interaction on dependent variables. Moreover, linear regression analysis was used to calculate the inhibitory concentration (IC_{50}) values. Correlation analysis was done to check the relationship between total phenolic contents and antioxidant activities. Statistical software SPSS for Windows 16.0 (SPSS; Chicago, IL, USA) was used to analyze all the data. Differences between means were separated using LSD test and *P*-value less than 0.05 was considered as statistically significant.

3. Results

3.1. Total phenolics and total flavonoids content of the tested spices

Both the spice type ($P < 0.001$, df = 4, $F = 8.969$.) and extraction solvent ($P = 0.048$, df = 1, $F = 5.352$) significantly affected values of total phenolics. Methanol extracts of *T. schimperi* and *L. adoensis* had higher total phenolic contents than aqueous extracts, whereas aqueous extracts surpassed methanolic extracts in total phenolic contents in *T. ammi*, *N. sativa* and *P. capense* (Figure 1A). The tested spices can be arranged in decreasing order of: *L. adoensis* > *T. schimperi* > *P. capense* > *N. sativa* > *T. ammi* in terms of the amount of total phenolic content in methanolic extracts. In aqueous extracts, the value of total phenolic content measured in *L. adoensis* was higher than *P. capense* and *T. schimperi* (whose values are more or less similar) followed by *T. ammi* and *N. sativa* with nearly similar values (Figure 1A).

Likewise, values of total flavonoids were affected by the spice type ($P < 0.001$, df = 4, $F = 31.461$) and extraction solvents ($P < 0.001$, df = 1, $F = 56.734$). Except in *P. capense*, methanol extracts of the tested spices had higher total flavonoids than aqueous extracts with values in decreasing order arranged as *L. adoensis* > *T. schimperi* > *T. ammi* > *N. sativa* > *P. capense* (Figure 1B). Total flavonoid content in aqueous extracts of the spices followed similar trend to total phenolics content measured in methanol extracts.

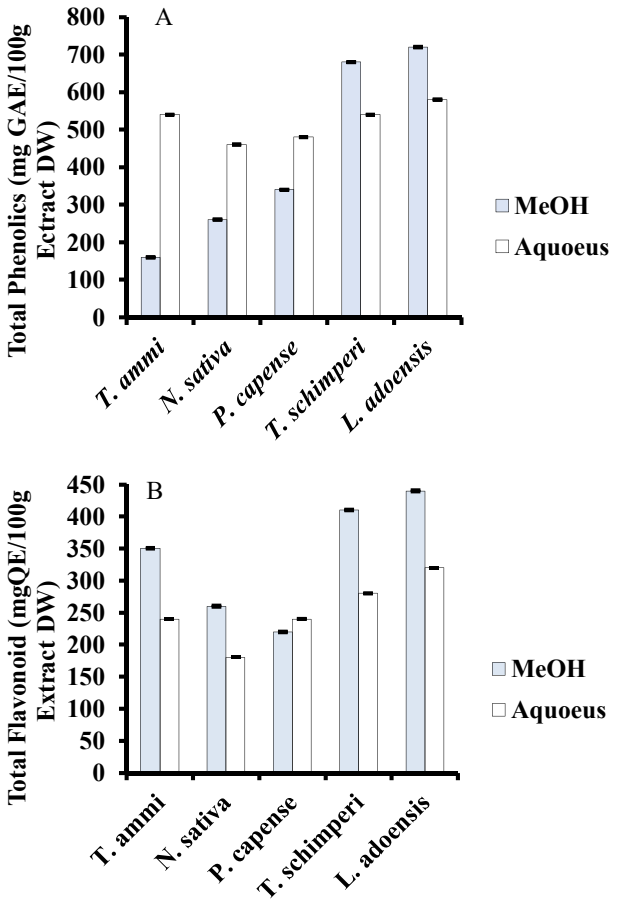

Figure 1. Values of Total phenolics (A) and Total flavonoids (B) Content. Values are Mean ± SE, n = 3.

3.2. Antioxidant capacity of the tested spices

3.2.1. DPPH· stable radical scavenging capacity

With regard to DPPH stable radical quenching activity of the methanol and aqueous extracts of different spices, a concentration dependent radical scavenging ability towards DPPH was observed. There was significant ($P < 0.001$, df = 6, $F = 4.577$) difference between antioxidant sources in DPPH scavenging potential at each concentration level examined. Some of the spices were on a par with standard antioxidant compounds, that is, BHT and AA (Figure 2 A and B). There was significant difference ($P < 0.001$, df = 6, $F = 336.284$) between extract concentrations in terms of DPPH radical scavenging potential. Extraction solvent used to get the extracts had also significantly impacted ($P < 0.001$, df = 1, $F = 65.212$) DPPH stable radical quenching activity. DPPH radical scavenging potentials of most spices' methanolic extracts were superior to aqueous extracts (Figure 2 A and B). The DPPH scavenging activity of methanolic extracts showed strong positive correlation ($r^2 = 0.980$, $P = .003$) with total phenolic contents. Likewise, the correlation between DPPH scavenging activity of aqueous extracts and total phenolic contents was strong ($r^2 = 0.945$, $P = .016$). Assessment by IC_{50} values also showed significant ($P < 0.001$, df = 4, $F = 25.041$) variation between spices in their DPPH scavenging activity by their methanolic extracts with values in decreasing order of scavenging activity: *L. adoensis* ($IC_{50} = 49.2 \pm 4.2$ μg/mL)>*T. schimperi* ($IC_{50} = 60.1 \pm 0.4$ μg/mL)>*P. capense* ($IC_{50} = 71.9 \pm 1$ μg/mL) >*T. ammi* ($IC_{50} = 74.4 \pm 2.7$ μg/mL)>*N. sativa* ($IC_{50} = 94.1 \pm 5.6$ μg/mL). Similarly, the IC_{50} values of aqueous extracts significantly ($P < 0.001$, df = 4, $F = 64.995$) varied between spices in scavenging

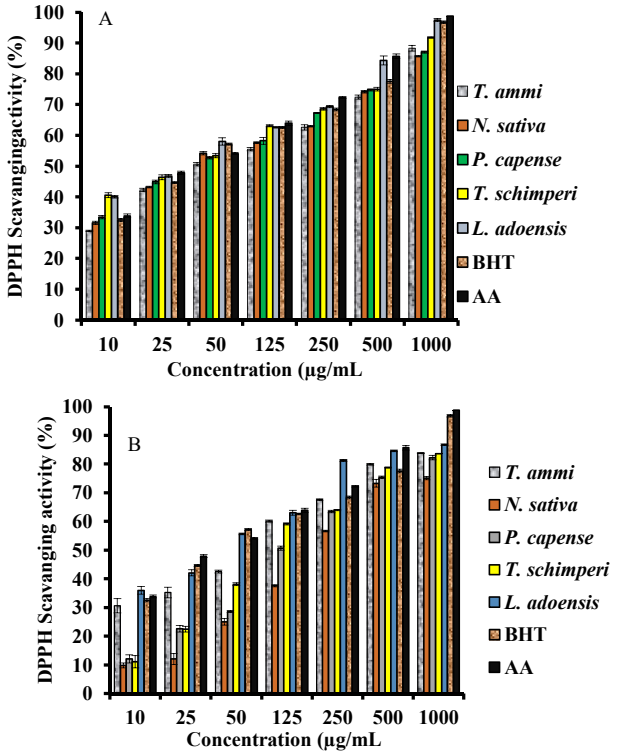

Figure 2. DPPH quenching capacity of (A) methanolic extracts and (B) aqueous extracts of the tested spices and standard antioxidant compounds. Values are Mean ± SE, n = 3.

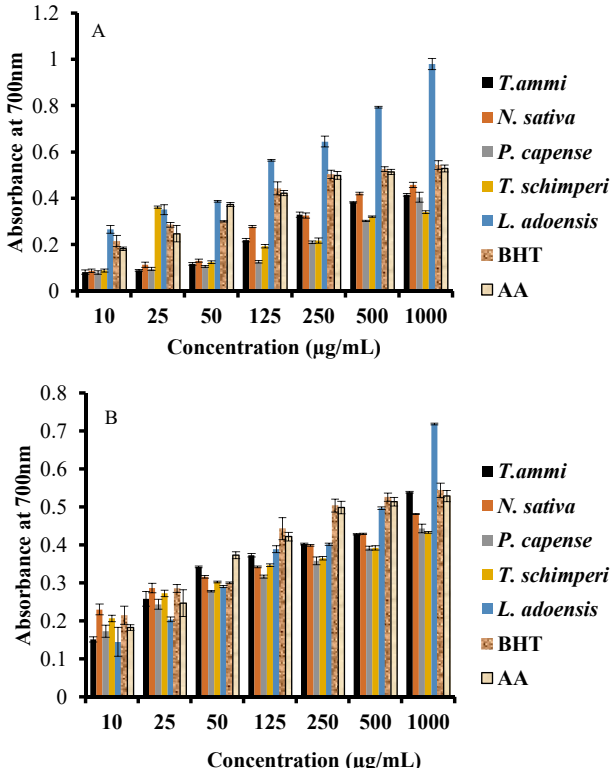

Figure 3. Reducing capacity of (A) methanolic extracts and (B) aqueous extracts of the tested spices and standard antioxidant compounds. Values are Mean ± SE, n = 3.

DPPH radical in decreasing order of their potential, *L. adoensis* (IC_{50} = 20.9±5 µg/mL)>*T. schimperi* (IC_{50} = 149.8 ± 3.1 µg/mL)>*P. capense* (IC_{50} = 205.5 ± 34.8 µg/mL)> *T. ammi* (IC_{50} = 323.2 ± 5.5 µg/mL)> *N. sativa* (IC_{50} = 330.2 ± 1.6 µg/mL). The scavenging activity of all spices, however, found to be lower than the standard antioxidant, ascorbic acid, with an IC_{50} value of 17.69 ± 6.63 µg/mL.

3.2.2. Fe^{3+} reducing capacity

The reducing capacity of the tested spices are presented in Figure 3 A and B. There was significant ($P < 0.001$, df = 6, $F = 29.977$) difference between antioxidant source in reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) at each concentration level examined. The reducing potentials of the spices varied significantly ($P < 0.001$, df = 6, $F = 67.465$) between extract concentrations. Extraction solvent used to get the extracts had also significantly impacted ($P < 0.001$, df = 1, $F = 14.758$) reducing potential of each spice whereby some spices had higher potential when extracted with methanol than aqueous, whereas the reverse is true for other spices. Among the tested spices, methanol and aqueous extracts of *L. adoensis* exhibited better reducing power than other spices, and at the highest concentration tested it was also found to be superior to standard antioxidants in its reducing capacity. Next to *L. adoensis*, *N. sativa* or *T. ammi* was more effective in reducing Fe^{+3} to Fe^{+2} depending on the extraction solvents used to extract them. Strong positive correlation was observed between total phenolic contents of aqueous extracts and ferric reducing capacity ($r^2 = 0.942$, $P = .017$). Similarly, positive correlation was found between ferric reducing capacity of methanolic extracts and total phenolic contents, but values are not significant ($r^2 = 0.620$, $P > 0.05$).

3.2.3. The Reactive Nitrogen Species (RNS) inhibition capacity

There was significant ($P < 0.001$, df = 6, $F = 199.968$) difference between antioxidant source in Reactive Nitrogen Species (RNS) inhibition potential at each concentration level examined. The RNS inhibition potentials of all spices were significantly lower when compared with standard antioxidant compounds, which are, BHT and catechin. Antioxidant capacity of BHT was on a par with catechin at the highest concentration, but appeared to be lower than catechin at the lower concentration levels. The RNS inhibition potentials of the spices varied significantly ($P < 0.001$, df = 6, $F = 222.414$) between extract concentrations and inhibitory effect appeared to increase in a concentration dependent manner. Extraction solvent used to get the extracts had also significantly impacted ($P < 0.001$, df = 1, $F = 15.470$) RNS inhibition potential of each spice whereby some spices had higher potential when extracted with methanol than aqueous, whereas the reverse is true for other spices (Figure 4 A and B). Though correlations are positive between RNS inhibitory effects of both methanol and aqueous extracts, they were insignificant ($r^2 = 0.683$, $P > 0.05$). Assessment by IC_{50} values also showed significant ($P < 0.001$, df = 4, $F = 143.373$) variation between spices in their RNS inhibition activity by their methanolic extracts with values in decreasing order of RNS inhibition activity: *L. adoensis* (IC_{50} = 597.2 ± 4.9 µg/mL) >*P. capense* (IC_{50} = 635.4 ± 4.9 µg/mL)> *T. ammi* (IC_{50} = 676.6 ± 1.69 µg/mL)> *T. schimperi* (IC_{50} = 768.2 ± 14.1 µg/mL)> *N. sativa* (IC_{50} = 797.5 ± 2.7 µg/mL). Similarly, for the aqueous extracts, the IC_{50} values significantly ($P < 0.001$, df = 4, $F = 92.821$) varied between spices in RNS inhibition potential in a decreasing order of their potential, *L. adoensis* (IC_{50} = 551.5 ± 28.9 µg/mL) >*N. sativa* (IC_{50} = 625.7 ± 0.82 µg/mL) > *T. ammi* (IC_{50} = 630.1 ± 17.9 µg/mL) >*P. capense* (IC_{50} = 674.9 ± 28.9 µg/mL) >*T. schimperi* (IC_{50} = 1436.1 ± 72.7 µg/mL). The activity of all spices, however, found to be lower than the standard antioxidants, catechin and BHT, with IC_{50} values of 386.02 ± 3.81 and 387.44 ± 4.2 µg/mL, respectively.

4. Discussion

4.1. Total phenolic and flavonoid contents

In the view of the up surging interest in the health benefits of the spices, we examined the radical scavenging and antioxidant properties of

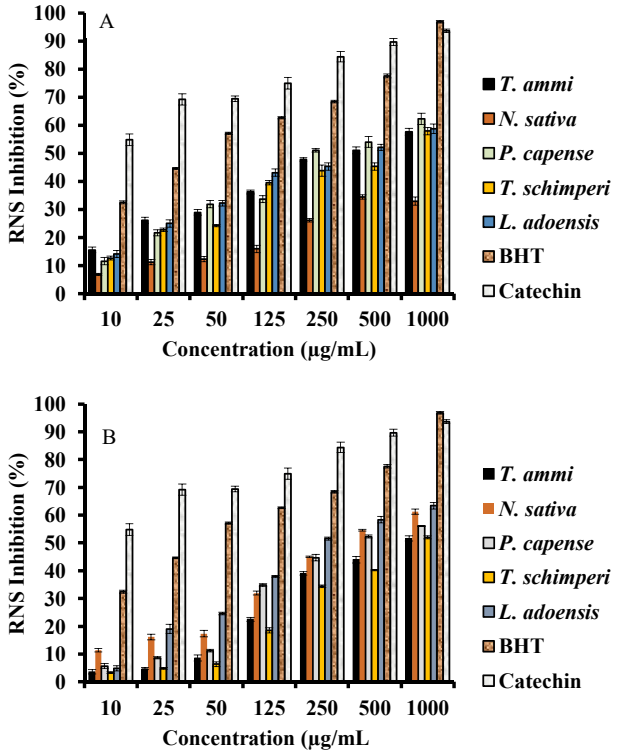

Figure 4. RNS inhibition capacity of (A) methanolic extracts and (B) aqueous extracts of the tested spices and standard antioxidant compounds. Values are Mean ± SE, n = 3.

the most commonly consumed spices in Ethiopia by *in vitro* standard methods. Prior to antioxidant activity assays, we analyzed the amount of total phenolics and total flavonoids contents in methanolic and aqueous extracts of the spices. Here, our interest was not to detect/quantify the complete phenolics profile and single out potent antioxidant compound(s), but gross quantification of phenolics that often are reported to have antioxidant property (Shan et al., 2005; Wojdyło et al., 2007; Charles, 2013; Srinivasan, 2014) in crude extracts. Results of chemical analysis showed that total phenolics and flavonoids contents varied between the tested spices' extracts obtained using the same extraction solvent, suggesting the presence of varied amount of extractable phenolic compounds by the solvent used. Most probably, variation in total phenolic contents of these spices is attributed to their difference in genetic makeup (Ebrahimzadeh et al., 2008).

In methanolic extracts, total phenolic content of *L. adoensis* was the highest followed by *T. schimperi*, *Piper capense*, *N. sativa* and *T. ammi*. The amount of total phenolic and flavonoid contents obtained from the same spice was also varied by the type of solvent used. For example, total phenolic content was higher in methanolic extracts of *L. adoensis* and *T. schimperi* than in aqueous extracts. This indicates that phenolic compounds in these two spices are extractable more by less polar solvent than by highly polar aqueous solvent. Whereas the reverse is true in *P. capense*, *N. sativa* and *T. ammi* as total phenolics measurement was higher in aqueous extracts than methanolic extracts. Solvents of different polarity have different potential of extracting compounds (Egigu et al., 2010). Excepting *P. capense*, total flavonoid content was also higher in methanolic extracts than aqueous extracts with the highest value measured for *L. adoensis* followed by *T. schimperi*, *T. ammi*, *N. sativa* and *P. capense*. In *L. adoensis*, *T. schimperi* and *P. capense* total phenolic content was 1.5–1.8-fold and 1.7–2.4-fold higher than total flavonoids content in methanolic and aqueous extracts, respectively. In methanolic extracts of *N. sativa*, total phenolics content was equivalent to flavonoids, whereas total phenolics content was lower than flavonoids in *T. ammi*. In aqueous extracts, however, values were 2-3-fold higher. Here it is noteworthy that in seed spices, the ratio of total phenolics to flavonoids content were lower as compared to leafy spices in methanolic extracts. This may be due to less extractability of some phenolic compounds, for example phenolic acids, that account for total phenolic values by methanol. It is also possible that since spice seeds were obtained from shop where they have been stored for some time, some phenolic compounds other than flavonoids that could have contributed to total phenolic content and able to be extracted by methanol might have been lost or transformed. In general, our test plants showed variation in their total phenolic contents. Results of the antioxidant assays were also varied between the test plants. From correlation analysis, we noticed strong positive correlation between total phenolic contents of our test plants and the different antioxidant assays, suggesting that differences of antioxidant capacities between our test plants can be ascribed to phenolic contents of the spices. Previously, several researchers (e.g., Kumar et al., 2014; Takao et al., 2015) showed the presence of strong correlation between antioxidant capacities and plants' total phenolic contents that confirms phenolic compounds as important contributors to antioxidant activities.

4.2. Antioxidant capacity of the tested spices

There are various methods that are used to determine the antioxidant potentials of plant extracts. The results to be obtained may of course be different from method to method due to the complexity of chemicals in the extracts leading to varying sensitivity to the test employed (Prior et al., 2005). In this study we used three tests viz. DPPH (1, 1-diphenyl-2--picrylhydrazine) radical scavenging assay, RNS inhibition assay and ferric reducing antioxidant power. The DPPH assay is a low cost method, which has frequently been used to evaluate the antioxidative potential of various natural products of plant origin (Molyneux, 2004; Mariod et al., 2009; Kim et al., 2011; Adefegha and Oboh, 2012). The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Mao et al., 2006). In our study, the methanol extracts of all the spices exhibited antioxidant property compared to the control (solution without plant extracts), which had 0% DPPH radical scavenging activity. At some concentration levels, some spices demonstrated better or equivalent antioxidative potential as reference antioxidants (BHT and AA). Generally speaking, however, scavenging activities of all spices' extracts and reference antioxidants increased with increasing concentrations, suggesting an increased ability of the extracts to donate hydrogen ions to the radical (Adebiyi et al., 2017). Comparison between the methanolic extracts of the tested spices based on IC_{50} values of DPPH scavenging activity showed that *L. adoensis* demonstrated the highest antioxidative activity followed by *T. schimperi, P. capense, T. ammi* and *N. sativa*. Similar trend was also observed in aqueous extracts. The observed trend of DPPH quenching activities corresponded to the amount of total phenolics measured in each spice. In our study, a strong and significant positive correlation was observed between total phenolic content and DPPH scavenging activity of both the methanol and aqueous extracts of the tested spices. The positive correlations obtained from our study support the hypothesis that DPPH radical scavenging activity is attributed to phenolic concentration. Previously, many researchers (e.g., Nickavar et al., 2008; Adebiyi et al., 2017; Savsani et al., 2020; Soulef et al., 2020) have shown strong positive correlation between antioxidant activity and phenolic contents in plant structure. The antioxidant capacity of phenolic compounds owes to their redox properties which help them act as reducing agents, hydrogen donors and/or singlet oxygen quenchers (Pietta, 2000).

In this study, the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) was measured by the methanolic and aqueous extracts of spices by the intensity of the resultant Prussian blue color complex which absorbs at 700 nm. The presence of antioxidants in the methanol and aqueous extracts of the spices were able to convert the oxidized form of Fe^{3+} into Fe^{2+}. The higher absorbance at high concentration indicates the strong reducing capacity. Results of this study showed that all spices' extracts exhibited a noticeable concentration-dependent reducing power though

reducing power was differed between spices and extraction solvents used. In most cases, methanol extracts had stronger ferric reducing antioxidant power than the aqueous extracts. Interestingly methanol and aqueous extracts of *L. adoensis* with absorbances of 0.98 and 0.72, respectively possessed more pronounced reducing capacity than that of BHT (absorbance, 0.543) and ascorbic acid (absorbance, 0.542) at 1000 μg/mL. The findings of reducing capacity indicate that the noticeable ferric reducing power of the extracts of the tested spices appear to be due to the presence of polyphenols in the extracts, which may exert their action by breaking free radical chain by donating a hydrogen atom (Duh et al., 1999). In our research, correlation analysis between total phenolic content of aqueous extracts and ferric reducing capacity was performed and a strong positive correlation ($r^2 = 0.942$, $P = .017$) was observed. However, the correlation between total phenolic content of methanol extracts and reducing power was relatively lower ($r^2 = 0.620$, $P > 0.05$). Correlation coefficient value of higher than 0.61 and lower than 0.9715 is considered as the high positive correlation (Thaipong et al., 2006).

Nitric oxide (NO*) is a reactive nitrogen species (RNS), which can be changed to stronger oxidant, peroxynitrite, upon reaction with superoxide anion radical (O_2^{*-}) (Nimse and Pal, 2015). Natural antioxidant compounds of plant origin compete with nitric oxide for super oxide oxygen so as to prevent the formation of peroxynitrite, which oxidizes a wide range of biomolecules (Nimse and Pal, 2015). In this study, both the methanolic and aqueous extracts of the tested spices significantly inhibited the formation of potent RNS when compared with the control (solution without extracts) that had 0% inhibition. Percent inhibitions of the spices increased with increasing extract concentrations, but significantly lower than the standard antioxidants (BHT and catechin). Based on the methanolic extracts, RNS inhibition activities of the tested spices were in the order of *L. adoensis* > *P. capense* > *T. ammi* > *T. schimperi* > *N. sativa*. On the other hand, RNS inhibition activity of *N. sativa* was second to *L. adoensis* though it appeared to be the last in its methanolic extract. This shows that antioxidant activity of a spice may vary by the type of solvent used to get extract. The values of RNS scavenging activity were moderately correlated ($r^2 = 0.683$, $P > 0.05$) with the total phenolic contents of the spices. Compared to the DPPH assay, the relatively lower correlation value observed between total phenolics content and RNS inhibitory potential may be due to variation of the assay methods used (Cai et al., 2004).

5. Conclusion

In the present study, we focused on total phenolic, total flavonoid contents and antioxidant activities of crude extracts from selected Ethiopian spices. Generally, the results of this study showed that all the tested spices have antioxidant property with their radical scavenging and reducing power. In this study, high to moderate positive correlations were observed between the total phenolic contents and antioxidant properties in different *in vitro* antioxidant assays. In all of the employed assays, a varying antioxidant activity was found among the spices studied, and *L. adoensis* demonstrated better activity than the rest of the spices, suggesting variation in antioxidant property among the spices emanated from the difference in the amount of total phenolic contents. As these spices are promising sources of natural antioxidant agents, further studies on isolation and characterization of the active antioxidant phenolic compounds should be carried out.

Declarations

Author contribution statement

Sasikumar Mahalingam, Oliyad Erba, Meseret C. Egigu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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