Total phenol contents, total flavonoid contents, antioxidant activities of Methanol extracts of Amomum Subulatum, Lippia adoensis, Coriandram sartivum, and Ruta chalepensis sold from Jigjiga market, Ethiopia

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Research Article

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Abstract

This research work aimed to analyze the total phenolic, flavonoid contents, and antioxidant properties of four selected medicinal plants *Amomum subulatum*, *Lippia adoensis*, *Coriandrum sativum*, and *Ruta chalepensis*. The Folin-Ciocalteu method to measure total phenolic content, and the aluminum chloride colorimetric method to measure total flavonoid content, the DPPH(1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP radical scavenging assay was used to measure the antioxidant activity. Results showed that *Lippia adoensis (*50 ± 0.23 mgGAE/g) and (20.5 ± 1.2 mgQE/g) *and Ruta chalepensis (*45 ± 0.76 mgGAE/g) and *(*17.3 ± 0.9 mgQE/g*)* have the highest phenolic and flavonoid content. The highest radical scavenging effect was observed in *Lippia adoensis* IC50 values 77.3 ± 2.8, 63.2 ± 2.3, and 15.8 ± 1.7 (µg/mL) DPPH, ABTS, and FRAP respectively. There is a positive correlation between total phenolic content, a flavonoid with antioxidant activity. Based on these results of the investigation, it could be concluded that *Lippia adoensis* is a rich source of phenolic compounds as natural antioxidants of high value.

1. Introduction

Oxidative stress plays a major role in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, and cardiovascular and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ or externally supplied through foods and/or supplements(1).

Plants are a rich source of natural bioactive compounds such as secondary metabolites and antioxidants(2). Plants contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g., phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins), nitrogen compounds (alkaloids, amines, and betalains), vitamins, terpenoids (including carotenoids), and other endogenous metabolites, which are rich in antioxidant activity(3).

Spices are herbs that contain complex mixtures of phytochemicals with characteristic odors and flavors. Spices are rich sources of phytochemicals, including phenolic compounds, which confer numerous health benefits including antioxidant, antidiabetic, and anti-inflammatory properties(4). Spices are abundant sources of compounds with strong antioxidant properties and can serve as substitutes for synthetic antioxidants in food systems to obtain additional health benefits. They are rich in antioxidant phenolic compounds(5).

Cancer is considered a major public health concern with a significant global impact on both developed and developing nations. Many bioactive chemotherapeutic and prophylactic agents have been isolated from plants, some of which have been used to improve efficacy by contributing a plethora of chemical structures that may yield novel bioactive compounds with pharmacological relevance(6). Some important natural products have been obtained from *Polycarpon tetraphyllum*, including total phenolic and

flavonoid contents and antioxidant, antimicrobial, and antiproliferative activities(7). Punica granatum Linne (Punicaceae) has been reported to possess anti-inflammatory properties(8).

Traditionally, *A. subulatum* is used to treat vomiting, abdominal pain, gastrointestinal (GI) infections, and rectal diseases(9). Large cardamom has been used as an antihyperglycemic, carminative, antiulcerogenic, antitussive, antibacterial, and cardioprotective agent to mitigate headache, bad breath, asthma, cough, liver diseases, anorexia(10), skin diseases, wounds, ulcers, bronchitis, fever, and hyperdipsia(11). Previous studies have reported carbohydrate, tannins, cardioactive glycosides, terpenes, flavonoids, alkaloids, saponins, and antibacterial activity against bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans* in methanolic extracts of Amomum subulatum (12). Another study reported four new chemical compounds geranil-3(10)-en-9-olyl octadec-9-enoate, geranil-3(10)-en-9-carboxyl- β-D-arabinopyranoside, geranilan-9-carboxy-a-L-arabinopyranoside and stigmast 5-en-3β-ol-3β-D-arabinopyranosyl-2'-(3'-methoxy)benzoate-30-octadec-9‴,12‴,15‴ trienoate(13).

C. sartivum has been traditionally used to treat bladder ailments, gastric and intestinal pain, muscular and rheumatic pain, insomnia, and diarrhea (14). *Coriandrum sativum* exerts pharmacological effects including antioxidant, antidiabetic, antimutagenic, anthelminthic, anticonvulsant, anxiolytic, and hepatoprotective effect(15), as well as essential oils in its leaves, stems, flowers, and fruits/seeds. Essential oils contain phenolic hydroxyl groups with antioxidant properties in the structure of their components(16).

Tunisian *Ruta chalepensis* is traditionally used to protect against various disorders, such as rheumatism, fever, mental disorders, dropsy, neuralgia, menstrual problems, convulsions, and other bleeding and nervous disorders(17). Previous studies have reported that the extraction of chloroform, ethyl acetate, and butanol isolated four compounds, namely, 7-hydroxy-6-methoxycoumarin, Kaempferol, Quercetin and quercetin 3-O-a-L-rhamno glucopyranosyl (Rutin), and revealed the presence of phytochemicals, carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, alkaloids, protein and/or amino acids, Resins, Lactones and/or esters, and tannins(18). Another study reported that alkaloids, tannins, saponins, flavonoids, and phytosterols and subjected to antibacterial activities of R. chalepensis (Fruit) against *S. aureus, P. aeruginosa, E. coli*, and *S. pneumoniae*(19). Another study also reported that Total phenol contents and flavonoid contents were 6.22 mgGAE/g and 6.59 mg QE/g respectively and revealed the presence of hesperidin (591.9 mg/g dry extract) and rutin (266.7 mg/g dry extract), antioxidant activities of DPPH scavenging (IC_{50}) 60 µg/mL and lipid peroxidation (IC_{50}) value of 16.9 µg/mL.(20).

In Ethiopia, *Lippia adoensis*, traditionally, the dried leaves are used for the preparation of spiced butter, which is used as a food flavoring agent and food preservative(21), and in skin diseases, including eczema and superficial fungal infections(22, 23), analgesic activity, and antipyretic activity(5). A previous study showed that Methanol extract showed 67.61 ± 9.89 mgGAE/g phenolic contents, 25.24 ± 0.43 mgQE/g flavonoid contents and antioxidant activities 10.96 ± 0.42 µg/ml DPPH, 123.97 ± 3.23 µg/ml iron

reducing power, 81.31 ± 15.94 µg/mL iron chelating activity(24) and antibacterial activity against the *S.aueres, P.aeroginosa, E.coli* and *S.typh*(25).

In general, the methods to determine the total antioxidant capacity are divided into two major groups: assays based on the single electron transfer (SET) reaction, displayed through a change in color as the oxidant is reduced, and assays based on a hydrogen atom transfer (HAT). which measure the activity of the antioxidant to scavenge peroxyl radicals, such as the total radical trapping antioxidant parameter (TRAP) assay, the oxygen radical absorbance capacity (ORAC) assay, and the luminol-chemiluminescence based peroxyl radical scavenging capacity (LPSC) assay. The ferric reducing antioxidant power (FRAP), α-tocopherol/ Trolox equivalent antioxidant capacity (aTEAC/TEAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays include electron transfer reactions (26).

Therefore, the objectives of the present study were to determine the TPC and TFC in the methanolic extracts of spices (*Amomum subulatum*, *Lippia adoensis*, *Coriandrum sativum*, and *Ruta chalepensis)* sold in the Jigjiga Market, Ethiopia, using the Folin-Ciocalteu reagent, aluminum chloride, and to determine their free radical scavenging activities using the DPPH assay, ABTS radicals, and their ferric reducing antioxidant power (FRAP) with the corresponding assay.

2. Material and methods

2.1. Study area description

The study area for the evaluation of total phenol content, total flavonoid content, and antioxidant activity of *Amomum subulatum, Lippia adoensis, Coriandram sartivum*, and *Ruta chalepensis* was obtained from the Jigjiga market in Ethiopia. Jigjiga is the capital city of the Somali Region of Ethiopia. It is located 629 km from Addis Ababa and approximately 100 Km East of Harar City. Geographically, it is located between the latitudes and longitudes of 9°21'N and 42°48'E, with an elevation of approximately 1,610 m above sea level (Fig. 1). In Jigjiga, the estimated total population was 106,414 in 2015 (27). The Jigjiga market is a popular trading center for a variety of goods, including medicinal plants, and is known for its diversity of plant species.

2.2. Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade. The chemicals and reagents included: 0.2 N Foline-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), Methanol (98%) from Loba Chemie (Mumbai, India); whereas acetone, n-hexane, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acids and Quercetin from Merck (Mumbai, India)., 6–hydroxy-2,5,7, 8-tetramethyl chroman-2-carboxylic acid (Trolox) and ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) from (Sigma-Aldrich, USA)

2.3. Apparatus and equipment

Electrical grinder (IAK–WERKE, Germany), electronic balance (Bosch, Germany), Whatman No. 42 filter paper (110 mm), graduated pipettes, micropipettes (Mumbai, India), refrigerator (Hitachi LR902T, USA), orbital shaker (GEMMY Orbital Shaker, VRN-480, Taiwan), Balance (Sartorius, Germany), rotary evaporator (Bibby RE200, Sterilin Ltd., UK) and UV/Vis spectrophotometer (Sanyo SP75, UK) were used in the study.

2.4. Collection of Plant materials

Fresh mature parts of A. *subulantum* (fruit), *Lippia adoensis* (leaf), *Coriandram sartivum* (fruit), and *Ruta chalepensis* (aerial parts) were collected in January 2023 from Jigjiga Market. The plant was authenticated by the botanist Mr. Meheret Berie (MSc.) in the Department of Biology at Jigjiga University. A voucher specimen (jju.12, 13, 14, 15) of the plant was deposited at the herbarium of the same department. The collected plants were washed thoroughly with tap and distilled water. The plant samples were first dried at room temperature for ten days under a plastic cover to avoid dust contamination, powdered using an electric grinder, and stored in clean polyethylene plastic bags until extraction.

2.5. Extraction of Plants

Powdered plant material (50 g) was then extracted with 250 mL of methanol by maceration using an enclosed glass bottle with occasional shaking at room temperature for 72 h. The methanol extracts were filtered through Whatman filter paper no.42 and concentrated under reduced pressure using a rotary evaporator at 64°C. The resulting crude extract was stored in the dark at 4°C until further analysis. Before use, the extracts were dissolved in methanol to prepare 10 mg/mL stock solutions.

2.6. Determination of total phenolic content

The total phenolic content (TPC) was quantified using the Foline-Ciocalteu method(11). A 1 mg/mL solution of the samples was prepared in 80% aqueous methanol. To 0.5 mL of the sample solution, 2.5 mL of Folin-Ciocalteu reagent, and 2 mL of 7.5% Na_2CO_3 solution were added, and the mixture was allowed to stand at room temperature for 90 min. A reagent blank was prepared in parallel using distilled water. A standard calibration curve for gallic acid in the range of 10–500 mg/L was prepared in the same manner. Absorbance was measured against the prepared reagent blank at 760 nm using a double-beam UV/Vis spectrophotometer. The total phenolic contents were calculated from the calibration curve using gallic acid as a standard, and the results were expressed as milligrams of gallic acid equivalents per gram dry weight of extract (mg GAE/g DW). All the studied samples were tested in triplicate for each extract, and the mean absorbance values were reported. The total phenolic content in all samples was calculated using the formula (Equ.1):

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

where TPC is the total content of phenolic compounds in mg/g plant extract in GAE dry extract, C is the concentration of gallic acid obtained from the curve (mg/L), V is the volume of extract in mL, m is the mass of extract in grams and DF = Dilution factor.

2.7. Total Flavonoid Contents

The total flavonoid content of the samples was determined using the aluminum chloride colorimetric method(2), with some modifications, using quercetin as the standard. Each sample solution (1 mg/ml) was prepared in methanol. Briefly, the sample solution (0.5 mL) was added to 10 mL of each volumetric flask containing 4 mL distilled water. Then, 0.3 mL of 5% NaNO2 was added to each test tube. After 5 min of incubation, 2 mL of 2% $AlCl_3$ was added. After 1 min, 2 mL of 1 M NaOH was added to each test tube and the volume was adjusted with distilled water. All tubes were incubated at room temperature for 30 min. The absorbance was measured at 415 nm. The concentration of flavonoids was expressed as milligram quercetin equivalent (QE) per gram of extract. All samples were analyzed in triplicate. The total flavonoid content of the *extracts* was determined using (Equ.2).

$$TFC = \frac{C.V.DF}{m} \quad (2)$$

where TFC is the total content of flavonoid compounds in mg/g plant extract in CE, C is the concentration of catechin obtained from the curve (mg/L), V is the volume of the sample solution (L), m is the mass of extract in grams, and DF = Dilution factor.

2.8. Radical scavenging activity by DPPH assay

The free radical scavenging capacity of the methanolic extract was determined using the DPPH assay according to the method of Brand-Williams(11), with some modifications. A 0.1 mM solution of DPPH was prepared in 80% methanol. A 0.5 mL solution of the sample was prepared in 80% methanol. A series of dilutions of the sample solution was prepared, and 1 mL of each dilution was mixed with 3.6 mL of the DPPH solution. An equal amount of methanol (0.5 mL) was used as a blank (control) with 3.6 mL DPPH solution. The % inhibition of both the standard and samples were calculated for each concentration, and graphs were plotted (% inhibition against concentration). From these graphs, IC_{50} values were calculated for the standard and extracts using (Equ. 3). The experiment was performed in triplicate.

2.9. Ferric reducing antioxidant power (FRAP) assay

Total antioxidant activity was determined using the FRAP assay in the extract following a modified method reported by Benzie and Strain(28). For the FRAP assay, Fresh FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$) at pH 3.6, 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ solution(11). The temperature of the solution was raised to 37°C before use. The FRAP reagent (200 µL) was added to 50 µL of the sample extract in triplicate. The mixture was incubated in the dark for 30 min at room temperature and the absorbance was measured at 593 nm using a spectrophotometer. The concentration of FRAP in the extract was reported as mg Trolox equivalent (TE)/g extract.

2.10. ABTS radical scavenging assay

2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was performed(11). The ABTS radical cation ($ABTS^+$) was generated by mixing the following solutions; 7 mM $ABTS^+$ solution with 2.45 mM potassium persulfate in ratio of the 1:1 (v/v) solution. The reagent was

incubated in the dark at room temperature for 12–16 h to complete the reaction. The ABTS solution was diluted with methanol to obtain an absorbance of 0.7 at 734 nm. Then, 10 µL of the extract (1 mg/mL) was mixed with 990 µL of diluted ABTS solution. A series of standards were prepared in the range (0–400 mg/mL) and incubated for 30 min at room temperature. The absorbance was then measured at 734 nm. The % inhibition of both standard and samples was calculated, and the concentration of ABTS content in the extract was reported as mg of trolox equivalent (TE)/g extract. The percentage of ABTS inhibition was calculated using the formula Equ.3;

2.11. Assaying methods

All absorbance measurements were performed using a UV/Vis spectrophotometer (Sanyo SP75, UK). All experiments were performed in triplicate unless otherwise stated.

The % inhibition for DPPH, FRAP, and ABTS assay was calculated according to the formula

$$\% \text{ inhibition} = \left[\frac{AB-AA}{AB} x100\backslash\%\right] (3)$$

where AB is the absorption of the blank sample, and AA is the absorption of the sample/standard extract. A calibration curve was obtained by plotting the% inhibition against the Trolox standard concentration.

2.12. Statistical analysis:

Experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. Data were analyzed using one-way analysis of variance (ANOVA), with a significance level set at $p < 0.05$. Suitable software packages, such as GraphPad Prism and SPSS, were used for statistical analysis.

3. Results and Discussions

3.1. Total phenolic contents (TPC) Amomum Subulatum

The total phenolic content of the extracts *of Amomum subulatum, Lippia adoensis, Coriandrum sativum*, and *Ruta chalepensis* was determined using the Folin-Ciocalteu assay by manipulating the regression equation of the gallic acid calibration curve. The concentration of phenolic compounds per extract was expressed as mg GAE/g DW. The results obtained from the assay are expressed as mean ± standard deviation of triplicate analyses and are presented in Table 1.

The highest total phenolic content was found in *Lippia adoensis* (leaf) (50 ± 2.1 mg GAE/g DW), then *Ruta chalepensis* (aerial parts) (45 ± 0.76 mg GAE/g DW), *Amomum subulatum* (fruit) (35 ± 0.8 mg GAE/g DW), and *Coriandrum sativum* (seed) (25 ± 0.2 mg GAE/g DW). These results imply that Amomum subulatum has a greater phenolic component concentration than other plant samples. The decreasing order of phenolic content was *Lippia adoensis* > *Ruta chalepensis* > A. *subulatum* > *Coriandrum sativum*.

Table 1
Total phenolic and flavonoid contents of different extracts of Amomum subulatum, Lippia adoensis, Coriandrum sativum, and Ruta chalepensis (mean ± SD, n = 3).

Sample	(TPC) (mg GAE/g DW)	TFC (mg QE/g DW)
Amomum subulatum	35 ± 0.8	15.2 ± 0.6
Lippia adoensis	50 ± 0.23	20.5 ± 1.2
Coriandram Sartivum	25 ± 0.2	8.6 ± 0.3
Ruta chalepensis	45 ± 0.76	17.3 ± 0.9

Phenolic compounds are secondary metabolites, that are predominant constituents in plants and are categorized as phenolic acids, flavonoids, tannins, curcuminoids, coumarins, lignins, and quinones(11). Flavonoids and phenolics also protect against cancer, cardiovascular and respiratory disorders, arthritis, and early aging. In addition to contributing to the antioxidant defense system of the body, they induce the production of protective enzyme systems(29). Several factors can be affected by the chemical nature of phytochemicals, the TPC values can be affected by the plant part used, extraction methods, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances(30).

The previous report revealed total phenolic content (TPC) of fresh leaves from *A. Subulantum* was reported to be 11.04 ± 0.2 mg GAE/g DW for the ethanolic extract and 6.87 ± 0.7 mg GAE/g DW for the aqueous extract. Three to five times higher TPC values were obtained when comparing the findings of this study on *Amomum subulatum* to those reported by Prakash *et al.*(2012). Another study reported the TPCs of *A. subulantum* obtained from fruits using different solvents: aqueous, cold methanol, hot methanol, cold ethanol, hot ethanol, hydro alcohol, ethyl acetate, and acetone. The TPC values reported were 24.99 ± 0.25, 20.94 ± 0.21, 9.18 ± 0.13, 13.17 ± 0.13, 12.18 ± 0.09, 24.75 ± 0.27, 13.09 ± 0.95, and 6.56 ± 0.17 mg GAE/g, respectively. This study revealed TPC values are higher than those reported values by Kanthlal *et al.*(2021). The total phenolic in the extract depended on the polarity of the solvent used in the extraction. The high solubility of phenolic compounds in polar solvents results in high concentrations of the extract(32). The hydroxyl groups of phenolic components may contribute to their scavenging ability and antioxidant activity. Since phenol is the major constituent of large cardamom fruit, it can be hypothesized that this may be the prime source of its acceptable medicinal value (31).

The previous study showed that Tunisian three coriander fruits varieties (Tunisian, Syrian, and Egyptian) coriander methanolic extract had a polyphenol content of (1.09 ± 0.02 mg GAE/g) than Tunisian (1.00 ± 0.06 mg GAE/g) and Egyptian (0.94 ± 0.05 mg GAE/g) ones. This value is almost 25 times greater than that reported by Msaada *et al.* (2017). Similarly, a study conducted by Wangensteen *et al.* (2004) reported the TPC of *Coriandrum sativum* seeds and leaves. They conduct different solvents such as ethanol, dichloromethane, ethyl acetate, butanol, and aqueous reported TPC values for seed extracts to be 0.15 ± 0.01, 0.09 ± 0.01, 1.89 ± 0.08, 1.16 ± 0.01 and 0.43 ± 0.00 g GAE/g DW respectively. For leaves

extracts, the TPC values with ethanol, dichloromethane, ethyl acetate, butanol, and aqueous were 0.36 ± 0.03, 1.89 ± 0.07, 5.45 ± 0.09, 1.42 ± 0.02, and 1.06 ± 0.20 g GAE/g DW respectively(34), these reports are higher than this study. This results for *C. sativum* revealed greater values than those reported by Sadoun *et al.* (2021). The result of this study, the ethanolic extracts of the leaves and leaves with steam were more potent than the ethanolic extracts of the leaves alone, the ethanolic extracts of the leaves with steam, and the methanolic extracts(35).

A study conducted in Ethiopia by J. M. Sasikumar, Erba, and Egigu, (2020) found that the methanolic extract of *L. adoensis* leaves had 720 ± 0.04 mg GAE/100 g extract DW, whereas the aqueous extract had 580 ± 0.08 mg GAE/100 g of dry weight extract. The current investigation found that the value reported in a previous study conducted in Ethiopia was significantly higher, approximately 11–14 times more than the results of the current study. In another study conducted in Ethiopia, the total phenolic content of *L. adoensis* leaf extracts ranged from 10.2 ± 2.2 67.6 ± 9.96 mgGAE/g. The order of total phenolic content, from highest to lowest, was observed as follows: aqueous: methanol (20:80, v/v) > methanol > water > acetone > petroleum ether extracts(36).

In a Tunisian study, Ouerghemmi *et al.*(2017) reported that in different parts of *Ruta chalepensis*, including leaves, stems, and flowers range between 0.2 -168.91 mg GAE/g (17). Another study was also reported from Jordan by Althaher, Oran, and Bustanji (2021), which revealed that the total phenolic content was 5.00 ± 0.005 mg GAE/ g from aerial parts of *Ruta chalepensis*(37). This value is higher than the previously reported value. Another study reported the total phenolic content in *Ruta chalepensis* from northern Tunisia. The results showed that decoction and ethanol extracts obtained from *R. chalepensis* leaf and steam were expressed in mg catechin equivalents per 100 g dry weight. In *R. chalepensis*, the comparison between decoction and ethanolic extracts was organ dependent. Stem decoction was characterized by higher polyphenol content (6.08 ± 0.37 mg Eq Cat/100 g DW) as compared to leaf decoction and ethanol extract (3.90 ± 0.3 and 2.73 ± 0.5 mg Eq Cat/100 g DW, respectively)(38). Solvent extraction affects the total phenolic content and antioxidant activity of edible plants(39).

In conclusion, the TPC values reported in this study provide valuable information on the antioxidant potential of plant extracts. The most important aspect of phenolic compounds is the fact that they may act as strong antioxidants *via* diverse mechanisms, namely hydrogen atom transfer, transfer of a single electron, sequential proton loss, electron transfer, or chelation of transition metals, all of which prevent the production of free radicals(30).

3.2. Total flavonoid Contents (TFC)

Flavonoids, including flavones, flavonols, and condensed tannins, are plant secondary metabolites, and their antioxidant activity depends on the presence of free OH groups, especially 3-OH. Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and upregulate and protect antioxidant defenses (40).

Total flavonoid content was determined by the complex formation between aluminum chloride with the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom from the flavone and flavonol groups. Quercetin was used as a standard for determining flavonoid levels because it is a flavonol group with a keto group on the C-4 atom and a hydroxyl group on the C- 3 and C-5 atoms(32).

In this study, the flavonoid content (TFC) of the four Spices (*Amomum subulatum, Lippia adoensis, Coriandram Sartivum*, and *Ruta chalepensis*) were found to vary between the samples, *Lippia adoensis* had the greatest total flavonoid contents, with a value of 20.5 ± 1.2 mg QE/g DW, followed by Ruta chalepensis (17.3 ± 0.9 mg QE/g DW), Amomum subulatum (15.2 ± 0.6 mg QE/g DW), and Coriandrum sativum (8.6 ± 0.3 mg QE/g DW). This suggests that, when compared to the other samples tested, *Lippia adoensis* contained a considerably higher level of flavonoids. shown in Table 1.

The mean total flavonoid content of *Amomum subulatum* seed extracts obtained in the present study was higher than those recorded in India(31). In the previous stud reported from *Lippia adoensis* in Ethiopia, the total flavonoid contents were expressed in (mgQRE/g) range from 6.6 ± 0.2 to 25.3 ± 0.3 and decreased in the order of acetone > aqueous: methanol (20:80, v/v) > methanol > water extracts(24). The total flavonoid content of *the Ruta chalepensis* oil extract was 34.09 ± 0.140 mg QE/g oil extract, which was higher than that in the present study(37). The recent study by Ouerghemmi *et al*., (2017) reported that TFC values of 129.69 mg CE/g *Ruta chalepensis*, respectively. Another study by (33) also reported that TFC values of the Syrian variety (2.51 ± 0.08 mg CE/g DW) followed by Egyptian (2.07 ± 0.05 mg CE/g DW) and Tunisian (2.03 ± 0.04 mg CE/g DW) fruit extracts of *Coriandrum sativum*. The TFC values show that the plants under investigation are a good source of flavonoids, which have a variety of biological actions. The results of this study are consistent with those of previous investigations on botanically related species, which found comparable TFC values.

3.3. Antioxidant activity results and comparisons

The antioxidant activity, determined by three different methods, namely DPPH, ABTS, and reducing power assays, is presented in Table 2. For the DPPH assay, the IC50 values for *Amonum subulantum, Lippia adoensis, Coriandram sartivum*, and *Ruta chalepensis* were 149.8 ± 4.6, 77.3 ± 2.8, 143.7 ± 4.2, and 118.6 ± 118.6 ± 3.5 µg/, respectively. For the ABTS assay, the IC50 values for *Amonum subulantum, Lippia adoensis, Coriandram sartivum*, and *Ruta chalepensis* were 114.5 ± 3.86, 63.2 ± 2.3, 111.8 ± 3.2, and 92.5 ± 2.6 µg/mL, respectively. For the ABTS assay, the IC50 values for *Amonum subulantum, Lippia adoensis, Coriandram sartivum*, and *Ruta chalepensis* were 24.5 ± 1.1, 39.8 ± 1.7, 23.2 ± 1.0, and 26.4 ± 1.2 mg TE/g, respectively shown in Table 2 and Fig. 2).

Table 2
Antioxidant activities of the four plant species were determined using three different assays: DPPH, ABTS, and FRAP.

	DPPH (IC50, µg/mL)	ABTS (IC50, µg/mL)	FRAP (mg TE/g)
Amomum subulatum	149.8 ± 4.6	114.5 ± 3.8	39.5 ± 1.1
Lippia adoensis	77.3 ± 2.8	63.2 ± 2.3	15.8 ± 1.7
Coriandram Sartivum	143.7 ± 4.2	111.8 ± 3.2	23.2 ± 1.0
Ruta chalepensis	118.6 ± 3.5	92.5 ± 2.6	20.4 ± 1.2

3.3.1. DPPH radical-scavenging

The DPPH method with the stable organic radical 1,1-diphenyl-2-picrylhydrazyl is used for the determination of free radical scavenging activity, usually expressed as IC50, which is the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. Lower IC50 values indicate higher antioxidant activity(41). *Lippia adoensis* and *Ruta chalepensis* extracts showed the highest capacity to neutralize this radical. Moreover, the obtained IC50 values were 77.3 ± 2.8 µg/mL and 118.6 ± 3.5 µg/mL, respectively. *Coriandram Sartivum* had the highest antioxidant activity with the lowest IC_{50} value of 143.7 ± 4.2 µg/mL, followed by *Amomum subulatum* with an IC_{50} value of 149.8 ± 4.6 µg/mL. In this study, the DPPH radical scavenging activity of the spice samples was in the following order: *Lippia adoensis > Ruta chalepensis > Coriandram Sartivum > Amomum subulatum.* The lower IC_{50} values of *Lippia adoensis* and *Ruta chalepensis* indicate that these plant species have a higher ability to scavenge DPPH radicals and protect against oxidative stress than *Amomum subulatum* and *Coriandram Sartivum (*Table 2 **and** Fig. 2). According to Msaada *et al.*(2017), the extract of the Tunisian coriander variety showed the highest capacity to neutralize this radical. The IC50 values obtained for the Tunisian, Syrian, and Egyptian varieties were 27.00 ± 6.57 lg/mL, 36.00 ± 3.22 lg/mL, and 32.00 ± 2.87 lg/mL, respectively. In comparison, the IC50 value for BHT was 18.00 ± 1.61 lg/mL. The previous study reported that antioxidant activities of the methanolic extract of coriander fruit different parts showed a concentration-dependent DPPH scavenging activity with IC50 of 32 ± 0.62 µg/mL in whole fruit, 42 ± 0.34 µg/mL in seed and 20 ± 0.25 µg/mL in the pericarp(39). Other reports showed that the DPPH scavenging activity of *C. sativum* leaf and stem aqueous extract had the lowest IC50 value (1335.0 ± 37.7 µg/ml, but also displayed the highest DPPH radical scavenging activity (IC50 = 2348.3 ± 184.1 µg/ml) among root extracts (42).

Prakash *et al.*, (2012) reported that *A. subulatum* leaves ethanolic and aqueous extract showed antioxidant activity with DPPH scavenging, IC50 value of 8.25 ± 2.0 µg/mL, and 21.6 ± 2.0 µg/mL respectively(10). Aftab and Rita (2018) determined the antioxidant activity of essential oils obtained from the fruits of seremna, varlangy, and sawney using three different methods: DPPH, ABTS, and FeCl3-reducing methods. The DPPH scavenging model, the IC50 value of the essential oil of the seremna, varlangy and sawney were found to be 172.3, 216.9 and 274.3 µg/mL, respectively, while in the ABTS

scavenging model, the IC50 value of the seremna, varlangy and sawney were found to be 27.96, 31.34 and 32.49 µg/ml respectively. The reducing power of the essential oil of seremna fruit, 1.453 was found to be higher than the varlangy, 1.011 and sawney, 1.001 at 200 µg/ml(43).

In the previous study reported by Ouerghemmi *et al*., (2017), the total antioxidant activities of *R. chalepensis* with DPPH scavenging range 23.73 ± 0.1 -80.77 ± 0.150 µg/mL, spontaneous and cultivated Ruta leaf and flower extracts both had higher scavenging activity than the stem extracts. This radical-scavenging ability was high, with IC50 values ranging from 23.73–30.69 3 µg/mL.

3.3.2. ABTS assay:

Similar to the DPPH assay, *Lippia adoensis* had the highest antioxidant activity with the lowest IC50 value of 63.2 ± 2.3 µg/mL, followed by *Ruta chalepensis*, with an IC_{50} value of 92.5 ± 2.6 µg/mL. *Amomum subulatum* and *Coriandram Sartivum* had higher IC_{50} values of 114.5 ± 3.8 µg/mL and 111.8 ± 3.2 µg/mL, respectively. The lower IC_{50} values of *Lippia adoensis* and *Ruta chalepensis* suggest that these plant species have a higher ability to scavenge ABTS radicals and protect against oxidative stress than *Amomum subulatum* and *Coriandram Sartivum* (Table 2 and Fig. 2**)**.

3.3.3. Ferric reduction (FRAP) assay

The reducing power of a bioactive compound may also serve as a significant indicator of its potential antioxidant activity(39). *Lippia adoensis* had the highest antioxidant activity with the highest TE value of 15.8 ± 1.7 mg TE/g, followed by *Ruta chalepensis* with a TE value of 20.4 ± 1.2 mg TE/g. *Amomum subulatum* and *Coriandram Sartivum* had lower TE values of 39.5 ± 1.1 mg TE/g and 23.2 ± 1.0 mg TE/g, respectively. The higher TE values of *Lippia adoensis* and *Ruta chalepensis* indicate that these plant species have higher ferric-reducing antioxidant power than *Amomum subulatum* and *Coriandram Sartivum (*Table 2 **and** Fig. 2*)*. According to Sriti *et al*.(2012), whole fruit showed a lower reducing capacity (EC50 = 780 g/mL) than the seed (EC50 = 680 µg/mL). The previous report that the concentration of *C. sativum* root extracts, the ethyl acetate extract was the highest FRAP value of 0.129 ± 0.007 mmol/g). For the leaf and stem, the highest FRAP value was observed in the dichloromethane extract (0.136 ± 0.008 mmol/g)(42). Ouerghemmi *et al*., (2017) reported that the total antioxidant capacity of R. chalepensis In the reducing power assay, spontaneous Ruta leaf and cultivated Ruta leaf and flower extracts demonstrated the highest reducing power (0.90 mg/mL, 0.92 mg/mL, and 1.10 mg/mL, respectively), followed by the stem extracts (1.96 mg/mL and 2.05 mg/mL for the spontaneous and cultivated provenances, respectively)(17).

In summary, our study provides new information on the antioxidant activity of four plant species, and our results are generally consistent with those of previous studies, although some differences could be attributed to differences in the extraction methods, solvent systems, and geographic origin. The difference in the antioxidant activity of different crude extracts depends on the total amount of phenols, flavonoids, and aromatic compounds present(44). Thus, these medicinal plants with higher antioxidant potentials can be used as an efficient source of natural antioxidants to treat various oxidative stress-

related problems, such as cancer and other cardiovascular disorders. By evaluating the results of this study using the DPPH, ABTS and FRAP assay, a quality control procedure could be designed and potentially used to develop a more effective protocol for investigating the antioxidant, anticancer, and phytochemical properties of medicinal plants.

Conclusion

In conclusion, the total phenolic and flavonoid concentrations of several extracts made from *Amomum subulatum, Lippia adoensis, Coriandrum sativum*, and *Ruta chalepensis* were examined in this study. The plant possesses a high amount of total phenolic and total flavonoid contents. In addition, DPPH, ABTS, and FRAP scavenging activity were found to be significantly correlated with the amount of TPC and TFC. *Lippia adoensis* and *Ruta chalepensis* extracts showed the highest capacity to inhibit ROS formation in both DPPH and ABTS assay at low concentrations. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract. These findings offer solid proof of the health advantages of desert plants and offer a promising combination of natural antioxidant phytochemicals.

Declarations

Ethics approval and consent to participate

All methods are carried out according to the institution's guidelines and regulations.

Consent for publication

Not applicable

Availability of data and materials

All data generated and analyzed are included in this research article.

Competing interests

The authors declare that they have no competing interests

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Funding is not applicable to this study.

Authors' contributions

AA - Study design, Literature search, data collection, data analysis, data interpretation, writing the manuscript.

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Figures

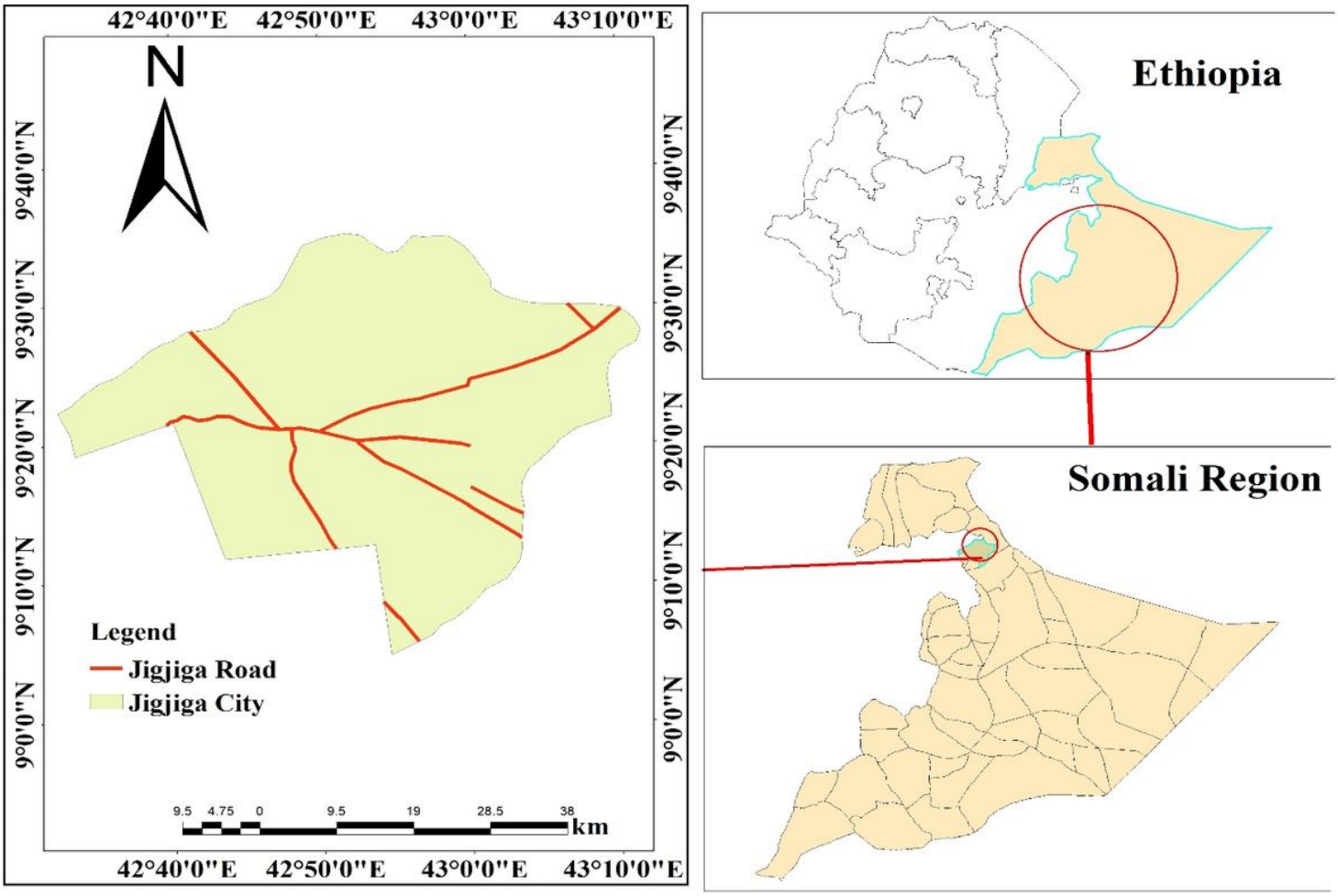

Figure 1

Map of the study area and sampling sites.

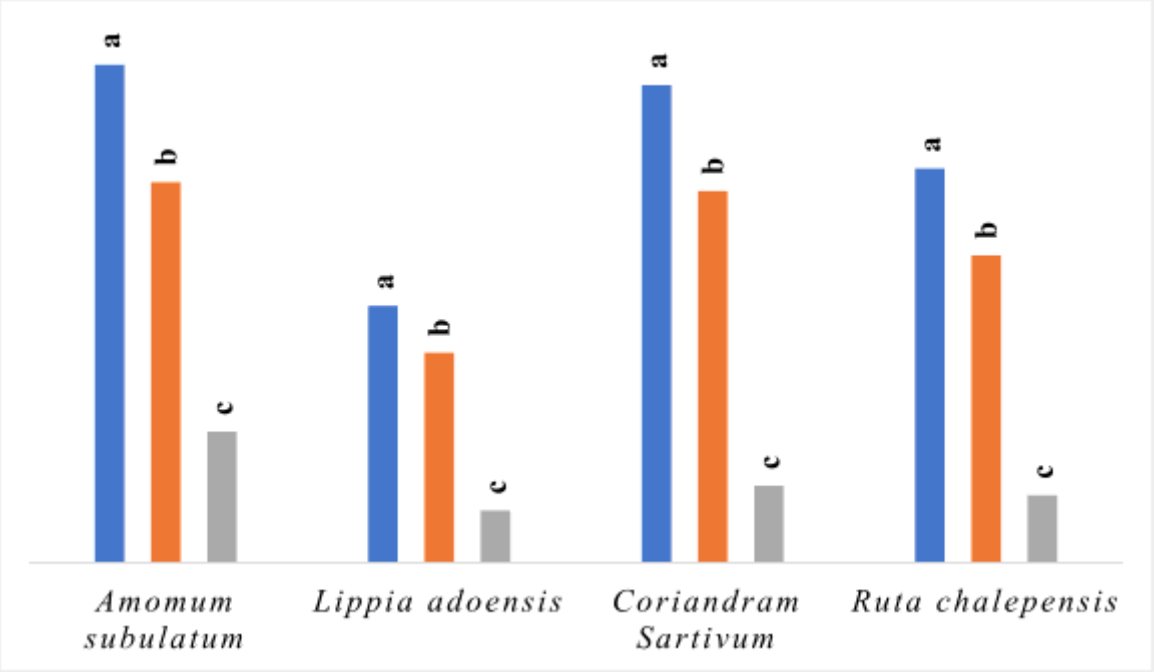

Figure 2

Antioxidant activity of plant extracts evaluated by (a) 1,1-diphenyl-2-picrylhydrazyl (DPPH), (b) 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS), (c) Ferric reducing antioxidant power assay (FRAP).