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Research Article
Studies on Antioxidant and Antimicrobial Activities of *Salvadora persica*

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Abstract

Background and Objective: *Salvadora persica* (*S. persica*) is a medicinal plant which has many traditional uses against infections and related disorders. The main objective of this study was to estimate total flavonoid and polyphenol contents, antioxidant and antimicrobial activities of ethanol extracts of root and stem parts of the *Salvadora persica* plant. **Materials and Methods:** The extraction was done using maceration method. Total phenolic content, total flavonoid content and antioxidant activity of *S. persica* extracts were evaluated using the Folin ciocalteu, the aluminum chloride colorimetric and reducing power (FRAP) assays, respectively. The agar well diffusion method was used for the determination of antibacterial activity. Results were subjected to analysis of variance (one-way ANOVA) by applying Duncan's *post hoc* test using SPSS. **Results:** Antioxidant activity of root and stem extracts of *S. persica* was expressed as ascorbic acid equivalent (206.593±17.5771 and 168.931±12.9975, respectively). Total phenol contents of root and stem extracts were also expressed in terms of gallic acid and gave values of 91.0 and 70.5 GAE/100 g of dry weight, respectively. About 40.528 and 2.6225 mg of QE per 100 g of dry weights of a sample were recorded for root and stem extracts when flavonoid contents of the extracts were determined, respectively. Antibacterial activity of both stem and root samples were assessed and *Staphylococcus aureus* was not inhibited by either of the two extracts. However antibacterial activity of root showed a positive result against *Streptococcus pneumoniae, Shiegella flexneri* and *Escherichia coli* (*E. coli*). The stem also gave similar result except it strongly inhibits *Shiegella flexneri*. **Conclusion:** *Salvadora persica* root and stem extracts were found to contain noticeable amount of total phenols and small amount of flavonoids which play major role in controlling oxidation. Furthermore, this study proves the effectiveness of *Salvadora persica* root and stem extracts for its excellent antioxidant and antimicrobial activities.

Key words: Polyphenol, percent inhibition, antimicrobial power, quercetin, gallic acid, ascorbic acid

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Many human diseases are initiated by free radicals. The natural defense of the human organs against free radicals is not always sufficient mainly due to the significant exposition to free radicals from different sources. In the modern world, substances such as cigarette smoke, environmental pollutants, ultraviolet radiation, certain drugs, pesticides, anesthetics and industrial solvents are contributing a lot. Therefore, the human body has to be protected against free radicals using antioxidants[1].

Antioxidants can be defined as a compound capable of inhibiting oxygen mediated oxidation of diverse substances from simple molecule to polymer and complex bio-systems[2-3]. It was also defined as the substance that when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance[4]. Two principle mechanisms of action have been proposed for antioxidants. The first was a chain-breaking mechanism, by which the primary antioxidant donates an electron to the free radical present in the system (e.g., lipid radical). The second mechanism involves removal of ROS/RNS initiators (secondary antioxidants) by quenching chain-initiating catalysts[5].

Traditionally people use different part of plants for medical purpose. Medicinal plant is defined as any plant with one or more of its organs containing substance that can be used for therapeutic purpose or which can be used as precursors for the synthesis of antimicrobial drugs, antioxidant, anti-infectious and anti-tumor activities[6]. Plants are the sources of medicines for many people of different age in many countries of the world, where diseases are treated primarily with traditional medicines obtained from plants. They have a wide activity range, according to the species, the topography and climate of the country of origin. They may contain different categories of active principles[7].

Herbal medicines have been widely used and form an integral part of primary health care of many countries and may constitute a reservoir of new antimicrobial substances to be discovered. Nearly all culture and civilizations from ancient times to the present day have depended fully or partially on herbal medicines because of their effectiveness, affordability, low toxicity and acceptability. The modern pharmaceutical industry itself still relies largely on the diversity of secondary metabolites in plants[8, 9].

Salvadora persica was used by the Babylonians some 7000 years ago. It was later used throughout the Greek and Roman empires and has also been used by ancient Egyptians and Muslims[10]. The main use of *S. persica* is as a tool for teeth, tongue and gum cleaning and has also been used to treat toothache[11]. The fresh root-bark and leaves have been used in folk medicine for the treatment of a wide range of human aliments such as cough, asthma, scurvy, piles, rheumatism, leprosy, gonorrhea, headaches and hepatic disorders[11].

Polyphenolic compounds have been reported to have a wide range of biological activities, many of which are related to their conventional antioxidant action. However, increasing scientific knowledge has highlighted their potential activity in preventing oral disease, including the prevention of tooth decay[12,13].

The phytochemical screening of the extracts revealed that the herbaceous parts of *S. persica*, contained carbohydrates, glycosides, sterols, terpenes, flavonoids, tannins and alkaloids but, are deprived of saponins, coumarins and anthraquinones[14]. Tannic acid, Sulfated compounds, benzyl isothiocyanate, liriodendron and salvadoracine are reported to have antimicrobial effects and help the healing of gum inflammation. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications like antioxidant, anti-inflammatory, laxative, analgesic and heart diseases[15-17.] The activities of the plant were investigated and the positive values of the plant in traditional medicines were evaluated. Therefore, this study will serve as preliminary resource in bioassay-based phytochemical investigations for coming researcher, can contribute to the delivery of better health care to the needy people and can help to maintain community's cultural and spiritual values passing to future generation.

MATERIALS AND METHODS

The root and stem of *S. persica* plant were purchased from a market in Bahir Dar city originally collected from Afar region during January, 2013. The plant was authenticated by natural database for Africa NDA with species ID 327. The root and stem parts were cut in to pieces, washed with tap water and dried separately at room temperature in shaded for three weeks. The dried samples were made in to small pieces by using pestle and mortal and then ground with a coffee grinder. About 35 g of dry plant powders were soaked in 350 mL of ethanol and stirred at 130 rpm using electrical shaker for 72 h. At the end of extraction, the crude extracts were filtered and filtrates were concentrated under reduced pressure using rotary evaporator at 37°C. The concentrated

solution was transferred to Erlenmeyer flask which was covered with aluminum foil and stored at room temperature in a dark place for further use.

Experimental procedure

Total phenolic content determination: Total phenolic content of *S. persica* extracts were determined using the Folin ciocalteu method[18]. With slight modification about 2.00 mL of each sample extract, in triplicate, was taken into a 50 mL conical flask, diluted with 45 mL of distilled water followed by addition of 0.25 mL Folin-Ciocalteu reagent and 0.5 mL of 7% sodium carbonate solution. The mixture was diluted to 100 mL and left for 30 min in dark. Finally absorbencies were measured at 750 nm using UV-vis spectrometer. Gallic acid was used as a standard and a blank was prepared by using distilled water instead of the samples and the standard.

Total flavonoid content determination: The total flavonoid content was determined by the aluminum chloride colorimetric assay[19] with few modifications. About 2.00 mL of the each sample extract, in triplicate, was taken and added to 4.00 mL of distilled water followed by an immediate addition of 0.30 mL of 5% $NaNO_2$ solution. After 5 min, 0.30 mL of 10% $AlCl_3$ solution was added and 5 min latter vigorous shaking, 2.00 mL of 1.00 M NaOH solution was added. Finally, the absorbance was taken at 510 nm. Quercetin was used as a standard and a blank was prepared by using distilled water.

Antioxidant activity determination using FRAP assay

Reducing power assay: The reducing power assay was used according to prior work[20]. To 2.00 mL of each of sample extract, 2.50 mL of phosphate buffer (0.20 M, pH = 6.60) was added followed by addition of 2.50 mL of 1% $K_3Fe(CN)_6$. This mixture was incubated at 50°C in water bath for 20 min. Then after, 2.50 mL of trichloroacetic acid was added. Subsequently, 2.50 mL of this mixture was taken and 2.50 mL of distilled water was added followed by addition of 0.50 mL 0.1% $FeCl_3$. Formation of green to blue colored solution was observed and the absorbance of this solution was taken at 700 nm. Ascorbic acid was used as a standard and a blank was prepared by using distilled water.

Antimicrobial property

Test microorganisms: Microorganisms used included 5 different bacterial strains, 3 strains of Gram-positive (*Staphylococcus aurous* (ATCC 25923), Methicillin Resistant *Staphylococcus aurous* (clinical isolate) and *Streptococcus pneumonia* (Clinical isolate) and two strains of Gram-negative: *Escherichia coli* (clinical isolate) and *Shigella flexneri* (ATCC 12022). The standard samples were obtained from Department of Biotechnology, University of Gondar and the clinical resistant pathogenic bacteria isolates were collected from Gondar College of Medicine and Health Sciences Hospital. The bacterial cultures were maintained in their appropriate agar slants at 4°C until further use.

Sample preparation and Susceptibility testing: About 50 g root and 20 g stem sample were extracted with 180 and 130 mL of ethanol at room temperature, respectively. The filtrates were obtained and put in oven at 40°C for 2 days to evaporate the solvent and the resulting extracts were kept for further use.

The agar well diffusion method was used in the susceptibility testing[21]. Bacterial suspensions of the test microorganisms were made in sterile normal saline and adjusted to the 0.5 McFarland's standard (appropriately prepared from barium chloride dihydrate and sulfuric acid). An inoculum was prepared by seeding the bacterial suspensions in a 100 mL petri dishes containing freshly prepared Mueller-Hinton agar medium. Then, agar wells were prepared on the inoculum by using a sterilized cork borer with 6 mm diameter. The wells were 4 mm deep and about 2.5 cm apart to minimize overlapping of zones. By using a micropipette, 100µl of the sample extracts, in triplicate, were carefully added to the wells and an antibiotic disc was dispensed with a sterile forceps onto the surface of the inoculated agar plate and pressed down to ensure complete contact with the agar surface. Crude extracts and the antibiotic disc were allowed to diffuse for about 40 min before incubation and then the plates were incubated in an upright position at 37°C for 24 h. Finally, the diameters of inhibition zones (clear transparent regions, including the well diameter) were measured in mm using a plastic ruler, which was held on the back of the inverted petri plate and the results were recorded. The antibiotic disc [Amoxicillin 25 µg (AML)] was used as a positive control while sterile, distilled water was used as a negative control.

Statistical analysis: All measurements were carried out in triplicates (n = 3) and values expressed are the mean of 3 repetitions±standard deviation (SD). Results were subjected to analysis of variance (one-way ANOVA) by applying Duncan's *post hoc* test using SPSS 20 software package (IBM Corporation) and graphs as well as regression coefficients were displayed using Origin 7 software (Origin Lab Corporation). The differences between means were determined by the least significant difference test and significance was defined at a confidence limit of $p<0.05$.

RESULT AND DISCUSSION

Determination of total polyphenol content (TPC): The results of the total phenolic content of *S. persica* root and stem extracts were measured in terms of gallic acid as a standard (mgGE per 100 g of dry weight of sample) in ethanol solvent extracts. Calibration curve was constructed by using different concentrations of (0.10, 0.50, 1.00 and 2.50 ppm) gallic acid and the corresponding absorbance as shown in Table 1.

The root extract was found to contain higher phenolic contents than the stem extract (91.0 and 70.5 mg of gallic acid equivalent/100 g of dry weight, respectively) as summarized in Table 2.

It was reported by Noumi *et al.*[22] that the stem has 44.3 mg/100 g, which is less compared to our results. Other work reported by Alali *et al.*[8] showed that the root of *S. persica* gave phenolic content of 1280 mg of gallic acid/100 g of dry weight. Biological activities of extracts vary depending on their chemical composition, which was determined by the plant genotype, geographical origin and agronomic conditions[23]. This might be the reason that different phenolic contents were reported by different researchers. The difference in activity between root and stem of *S. persica* extracts might be due to the accumulation of more phytochemicals in the root which are responsible for defense mechanism, in the contrary, exposure to sun light increases evaporation of chemicals from the stem.

Determination of flavonoid content (TFC): The contents of total flavonoids were quantified in terms of quercetin standard with $AlCl_3$ reagent[21]. The calibration curve was constructed by using different concentrations of quercetin (0.50, 1.50, 2.50, 3.50, 4.50 and 5.50 ppm) versus the corresponding mean absorbance obtained from UV-vis spectrophotometer. The reading of absorbance for each concentration is given in Table 3.

From the result, absorbance of root and stem extracts were recorded as 40.528 and 2.623 mg/100 g of quercetin, respectively. The maximum flavonoid content was found in the root extract of *S. persica*. Generally, as show in Fig. 1 and Table 4, the root contributes more antioxidant capacity, polyphenol and flavonoid contents than the stem extracts of *S. persica*.

Antioxidant determination using reducing power (FRAP) assay: The ability of the plants extracts to reduce ferric ions was determined using the FRAP assay. The FRP method measured the ability of an antioxidant to donate electron to Fe (III) resulting in the reduction of Fe^{3+}/ferricyanide complex to Fe^{2+} complex. The solutions of ascorbic acid standard at different concentrations were used to measure the reducing power of the extracts (Table 5). The equation y = 0.0119x-0.00035 was generated from the calibration curve, where y is the absorbance at 700 nm and x is concentration of ascorbic acid in ppm.

Similarly, four different concentrations of the root and stem extracts were examined and their absorbance were recorded and constructed as shown in Fig. 2 and Table 6. All extracts possessed the ability (either strong or weak) to reduce Fe^{3+}/ferric cyanide complex to the ferrous form. Figure 2 shows that absorbance increases with increasing concentration of both root and stem extracts of *S. persica*.

Table 1: Concentration of gallic acid and the resulting absorbance

Concentrations of gallic acid (ppm)	Absorbance
0.1	0.0147±0.008
0.5	0.0663±0.025
1	0.1343±0.012
2.5	0.2750±0.003

Data is expressed as mean of 3 determinations ±SD

Table 2: Absorbance of root and stem extracts *S. persica* per 100 g of dry weight of gallic acid

Plant parts	Average-absorbance value	GAE (mg/100 g)
Root	0.152±0.0095	91.0
Stem	0.124±0.0085	70.5

Mean±SD

Table 3: Concentration of standard quercetin and the corresponding absorbance

Concentrations (ppm)	Absorbance
0.50	0.062±0.003
1.50	0.076±0.008
2.50	0.087±0.003
3.50	0.097±0.001
4.50	0.128±0.005
5.50	0.149±0.006

Data is expressed as mean of 3 determinations ±SD

Table 4: Different concentration of ascorbic acid and the resulting absorbance

Concentrations (ppm)	Absorbance
4	0.048±0.0046
12	0.141±0.0139
20	0.243±0.0165
28	0.331±0.0161

Data is expressed as mean of 3 determinations ±SD

Table 5: Different concentration of root and stem extracts and their corresponding absorbance

Concentrations (ppm)	Absorbance	
	Root	Stem
16	0.087±0.00458	0.07±0.01389
32	0.154±0.00416	0.117±0.00755
48	0.213±0.00300	0.188±0.01060
64	0.326±0.01002	0.263±0.00917

Data is expressed as mean of 3 determinations ±SD

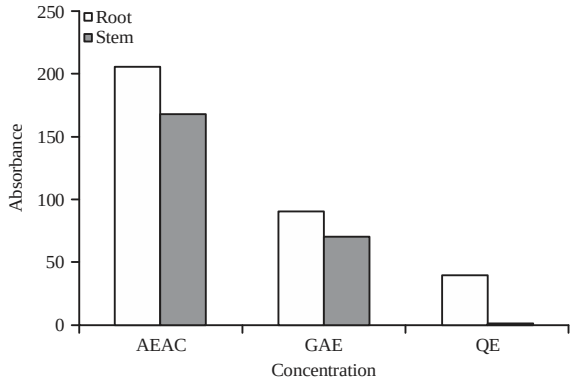

Fig. 1: Comparisons of the total flavonoid contents of the root and the stem extracts
AEAC: Ascorbic acid equivalent antioxidant content, GAE: Gallic acid equivalents, QE: Quercetin equivalents

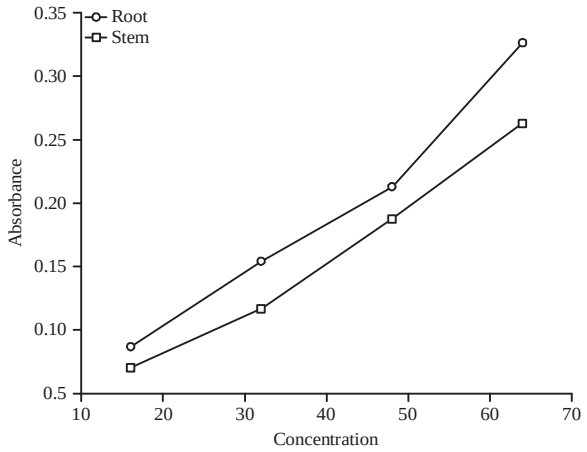

Fig. 2: Concentration (ppm) of root and stem extracts of *S. persica* versus absorbance

Table 6: Ascorbic acid equivalent antioxidant capacity value of the root and stem of *S. persica*

Parts of plant	AEAC (mg/100 g)
Root	206.593±17.577
Stem	168.931±12.998

Data is expressed as mean of 3 determinations ±SD

Table 7: Different bacterial strains and inhibition zone by root and stem extracts of *S. persica*

	Diameter of inhibition zone(mm)	
Bacteria used for test	Root	Stem
Staphylococcus aurous (ATCC 25923)	-	-
Staphylococcus aureus (MRSA)	-	-
Streptococcus pneumonia	10.33±1.5752	11.00±1.0000
Shigella flexneri (ATCC 12022)	10.00±2.6458	-
Escherichia coli	14.67±1.1547	15.67±0.5774

Data is expressed as mean of 3 determinations ±SD

Moreover, it can be seen from the result that the root extract exhibited better activity than equal amount of stem extract.

FRAP method is based on specifically the ability of a species having antioxidant property to donate electron to ferric complex and changing it to ferrous complex. Upon addition of potassium ferric cyanide to a solution containing sample and buffer, ferrocyanide was believed to be formed by taking electron from the extract. After incubation at 50°C for 20 min, trichloroacetic acid was added to stop the reaction. Prussian blue color which increases intensity with increasing concentration of the sample was observed after ferric chloride was added. The color change was due to formation of Fe^{2+}/Fe^{3+} complexes. Antioxidant capacity of the root of *S. persica* has greater than the stem extract.

This result implied that the potential antioxidant capability in *S. persica* may be attributed to in part from the antioxidant vitamins, phenolics, tannins and anthocyanin compounds present in this plant. Phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major contributors to the antioxidant capacities of most medicinal plants. In addition, the number and position of hydroxyl group of phenolic compounds also govern their antioxidant activity.

Antimicrobial activity determination: Root and stem extracts were tested against different bacteria namely: *Staphylococcus aureas*, *Streptococcus preumonoid* and *Shigella flexneri* and the results are summarized in Table 7.

Root and stem both show antimicrobial activity against different bacteria strains resulting almost similar inhibition zone. The root extracts demonstrated significant activity against *Shigella flexneri* but not stem extract. The inhibition of *Staphylococcus aureus* using methanol and aqueous stem extracts of *S. persica* has been reported[17,21]. Another study also showed the activity of the root and stem extracts against *Staphylococcus aureus*, *Streptococcus mutans*, *E. coli* and *Pseudomonas aeruginosa* in decreasing order[24]. But in this study, the ethanol extracts of the root and stem of *S. persica* were found inactive against *Staphylococcus* strains. However, the ethanol extracts exhibited greater inhibition zone against *E. coli* than reported[25]. Several factors may contribute for the difference. Temperatures, soil type, availability of nutrients in the soil and other environmental factors have a significant effect on the concentration of phytonutrients. However, these findings provide scientific evidence and confirmed the promising potential of this plant to use for medicinal purposes and support their traditional uses in folk medicine.

CONCLUSION

As it was exhibited, antioxidant power of the root was stronger than the stem extracts. Not only antioxidant power

but also polyphenol, flavonoid contents and antimicrobial activity of root was dominant over the stem of *S. persica*. Similar antimicrobial activities were investigated between the root and stem extracts. Therefore, it is not reasonable to correlate directly antioxidant activity, polyphenol and flavonoid contents with antibacterial activity. Extracts with higher antioxidant activity and high polyphenol or flavonoid concentration may or may not inhibit bacteria. The findings of this study support the view that the traditional practices of using the *S. persica* plant to treat several health problems could be attributed to the antioxidant and antibacterial activities of the plant. Therefore, the results presented here would encourage the use of *S. persica* plant as natural supplement in the management and control of human aliments caused by free radicals and pathogenesis of some diseases.

SIGNIFICANCE STATEMENTS

The results of this study could help to preserve indigenous knowledge and conserve biodiversity through preservation and documentation. Since the majority of the Ethiopian people rely mainly on traditional medicine to satisfy their primary health care need. The findings of this work are expected to serve as stepping stone for researchers to conduct further investigation.

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