

Antioxidant, Antimicrobial Activities and HPLC Quantitative Analysis of Some Sudanese Medicinal Plants

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Abstract

The objective of this study was to evaluate the antimicrobial and antioxidant activities and to determine some of bioactive components of ethanolic extract of *Adansonia digitata* fruit pulp, *Hibiscus sabdariffa* flower, *Hyphaene the baica* fruit pulp and *Ziziphus spina Christi* fruit pulp. The ethanolic extracts of the plants were tested against four bacterial strains (*Escherichia coli*, *Pseudomonasaeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*) and two fungal species (*Candida albicans* and *Aspergillus niger*) using disc diffusion method. Different plants extracts showed variable activity against all the tested microorganisms. The plant extracts were evaluated for their antioxidant activities using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assays. The highest result of antioxidant activity by DPPH and ABTS assays was observed in *Adansonia digitata* fruit pulp extract (77.4%) with IC_{50} of $0.273 \mu\text{g mL}^{-1}$ and *Hyphaene the baica* fruit pulp extract (79.02%) with IC_{50} value of $0.0808 \mu\text{g mL}^{-1}$. The identification and quantification of ascorbic acid, gallic acid and quercetin in the samples ethanolic extracts was performed by a HPLC-PDA method. These compounds have been detected in all the tested extracts.

Keywords: HPLC-PDA; Antioxidant Activity; Antimicrobial Activity; Sudanese medicinal plants; Bioactive compounds

Introduction

Plants play an essential role in our life, as a source of diet and medicinal agents^[1,2]. Sudan is one of largest countries in Africa with a wide genetic diversity of plants exist^[3,4]. Many of the herbal products are used extensively in Sudan as a traditional medicine for the treatment of several diseases. Different parts of plant such as, fruit, seeds, parks, stems, roots, peel, leaves and flowers are used as a constituents of herbal medicines^[1,5]. Primary and secondary metabolites are the two main constituents which are produced by plants^[6]. Primary metabolites such as sugars, proteins, lipids, chlorophyll and starch are directly involved in growth, development and production of plants^[7]. Secondary metabolites are the most valuable phytochemicals of plant which have an essential role in the protection of plants from attack by insect, herbivores and pathogens^[8,9]. Secondary metabolites include the phenolic compounds, flavonoids, terpenes, alkaloids, steroids, tannins, saponins, glycosides, flavonoids, etc. The pharmacological activities of plants are attributed to the secondary metabolites^[10]. Phenolic compounds represent the most abundant secondary metabolites in the plant^[11]. In human they have health promoting effects, such as anti-inflammatory anti-microbial, anti-cancer activity antiviral, anti-allergic and anti-platelet^[12,8]. It has been reported that phenolic compounds exhibit their protective and disease-preventing roles through their antioxidant activities^[13]. The reactive oxygen species (ROS) are in general linked

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to the most disease in human and are implicated in progression of cancer^[14,1]. These ROS can oxidize most bio-molecules such as DNA, protein, lipid and carbohydrates^[1]. Phenolic compounds as antioxidants play a significant role in scavenging free radical such as ROS, chelate metal catalyst and inhibit oxidase and hence prevent body from diseases^[10,13,15].

Ascorbic acid, gallic acid and quercetin are natural bio-active products which known to have biological activities including; antioxidant, anticancer, anti-inflammatory, anti-fungal and antiviral activity^[16-19].

The resistant of microorganisms to the available antibiotics is an emergent problem and worldwide issue^[20,21]. The natural phenolic compounds are effective in treatment of microbial infections. In Sudan several plants parts are used frequently in diseases treatment such as Roselle (*Hibiscus Sabdariffa*), baobab (*Adansonia digitata* L.), Doum (*Hyphaene thebaica* L. Mart) and jujube (*Ziziphus spina-christi* L.).

Roselle (*Hibiscus Sabdariffa*) is herbaceous shrub of family Malvaceae. It is a rich source of vitamins and bioactive compounds such as organic acids, phytosterols and polyphenols. It is used in many countries in treatment of hypertension, fevers, abscesses, cancer, cough and neurosis^[4,13].

The African baobab (*Adansonia digitata* L.) is a member of the Bombaceae family. Different parts of this tree are used in traditional medicine and as foodstuffs in many countries in Africa. It is well known to contain terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids. The various parts of plant are used to treat several ailments such as diarrhoea, malaria, anaemia, fever and microbial infections^[21].

The doum palm (*Hyphaene thebaica*) is a type of palm tree with edible fruit which belongs to the mint family (*Arecaceae*). The aqueous extract of doum fruits is rich in polyphenolic compounds and showed an anticancer and antioxidant activities since it contains substantial amount of their water-soluble phenolic contents^[22].

Jujube (*Ziziphus spina-christi* L.) is plant included in Rhamnaceae family. The plant extract is a promising natural product in the development of phytomedicines against drug-resistant infections^[23].

To investigate the quality and efficacy of these folk medicines, an effective analysis of their chemical constituents is required. Therefore, this research is aimed to evaluate the antimicrobial and antioxidant activity in ethanolic extract of *Adansonia digitata* fruit pulp, *Hibiscus sabdariffa* flower, *Hyphaene thebaica* fruit pulp and *Ziziphus spina Christi* fruit pulp growing in Sudan. Moreover quantitative analysis of some active compounds (ascorbic acid, gallic acid and quercetin) using HPLC–PAD detector was performed.

Materials and Methods

Standards, reagents and materials

Ascorbic acid (98.7%) was purchase from Supelco (Bellefonte, USA). Gallic acid (%) and quercetin (%), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were from Sigma Aldrich (St. Louis, MO, USA). Acetic acid glacial (99.5%) was from Scharlau (European Union). Acetonitrile HPLC grade (99.9%) and ethanol (99.8%) were from Duksan (Sinwonro, Korea). Potas-

sium persulfate (98%) and dimethyl sulphoxide were obtained from SDFCL (Mumbai, India).

Sample preparation and extraction

The edible parts of plant materials (*Adansonia digitata* fruit, *Hibiscus sabdariffa* flower, *Hyphaene thebaica* fruit and *Ziziphus spina Christi* fruit) were purchased from local market (Khartoum, Sudan). The plants materials were ground to a course powder using a pestle and mortar. Hundred gram of powder of each plant was extracted by ethanol at room temperature for 48 hours. After filtration, the extracts were vacuum concentrated.

Test strains and culture media

Standard strains of microorganisms were used in this study and were obtained from Medicinal and Aromatic Institute of Research, National Research Center (Khartoum, Sudan). The bacterial species used were the Gram-negative bacteria; *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the Gram-positive bacteria; *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Fungal species were *Candida albicans* (ATCC 7596) and *Aspergillus niger* (ATCC 9763). Bacteria organisms were grown in Mueller Hinton Agar and fungi organisms were grown in Sabouraud Dextrose Agar. The concentration of bacterial suspensions was adjusted to 108 cells mL⁻¹, and that of fungal suspensions to 107 cells mL⁻¹.

Preparation of culture media

Mueller Hinton agar was prepared by weighing 83 g of it then dissolved in 1.0 L of distilled water and allowed to soak for 10 minutes. The medium was placed in water bath to dissolve, swirled to mix and sterilized by autoclaving for 15 minutes at 121°C, cooled to 47°C and mixed well then poured into sterile Petri dishes.

Sabouraud Dextrose agar was prepared by dispersing 62 g of agar in 1.0 L of water and soaked for 10 minutes then swirled to mix. The culture media was sterilized by autoclaving for 15 minutes at 121°C, cooled to 47°C and mixed well then poured in to sterile Petri dishes.

Antimicrobial and antifungal analysis

Assay for antibacterial activity: Antibacterial activity of extracts was evaluated by the disc diffusion method^[24]. Extracts solutions (100 mg mL⁻¹) were prepared by diluting with 5.0% dimethyl sulfoxide (DMSO). The test microorganisms were seeded into respective medium by spread plate method. After solidification, filter paper discs with a diameter of 6.0 mm were impregnated with 10 µL of crude extracts followed by drying off. Antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 h at 37°C. Diameters of clear zone of inhibition produced around the discs were measured and recorded.

Bioassay for antifungal activity: The same method described for bacteria was adopted. To test antifungal activity, Sabouraud Dextrose Agar was used. The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for *Aspergillus niger*.

Determination of antioxidant activity

DPPH radical scavenging assay: The DPPH radical scavenging activity was determined according to the method of Shimada *et al.*^[25] with some modification. Briefly, 10 μL of sample extracts (5.0 mg mL^{-1} in DMSO) were transferred to a 96-wells plate and then 300 μL of DPPH solution in ethanol (0.5 mM) has been added. The mixtures were allowed to react for 30 min at 37°C. After incubation, decrease in absorbance was measured at 517 nm using multi-plate reader Spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. Ascorbic acid (2.0 mg mL^{-1}) was used as standard and all tests and analyses were run in triplicates. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

ABTS radical scavenging activity

ABTS radical scavenging activity of the extract was determined according to Re *et al.*^[26].

The ABTS⁺ cation radical was produced by the reaction between 5.0 mL of 14 mM ABTS solution and 5.0 mL of 4.9 mM potassium persulfate solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. Aliquots of 10 μL plants extracts (5.0 mg mL^{-1} in DMSO) have been homogenized with 300 μL of ABTS⁺ cation solution and allowed to react for 6.0 min then their absorbance were recorded at 734 nm. Ascorbic acid (2.0 mg mL^{-1}) was used as standard and all tests and analyses were run in triplicates. The inhibition percentage of ABTS⁺ radical was calculated using the following formula:

$$\text{ABTS scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

High performance liquid chromatography (HPLC) analysis:

The separation of ascorbic acid, gallic acid and quercetin in the plants ethanolic extracts were carried out on Waters HPLC with PDA detector (Waters, Milford, MA). The instrument equipped with a Waters alliance 2695 gradient separations module equipped with auto sampler and column oven, a Waters 2996 Photodiode array detector. All data were processed using Empower software (Waters, Milford, MA). The chromatograph-

ic separation was carried out under gradient conditions using a Shimpack VP-ODS (250 mm \times 4.6 mm, 5 μm) column (Shimadzu Corporation, Kyoto, Japan) at ambient temperature. The mobile phase was water containing 5.0% acetic acid (A) and acetonitrile (B) and the gradient program was as follows: was 90% B (0-5.0 min), 40 % B (6.0 min), 40% B (6.0-13 min), 90% (15 min) and 90% B (15-20 min). The flow rate was set at 1.0 mL min^{-1} and injection volume of 20 μL . The wavelength of PDA detector was in range of (200-450 nm). An aliquot of 10 mg of plant ethanolic extract was dissolved in a 3.0 mL mixture of acetonitrile and water (50: 50 v/v) and identified by comparison of the retention time in chromatogram with standard ascorbic acid, gallic acid, and quercetin. Data analysis was done using Empower software.

Results and Discussion

Antimicrobial and antifungal activity

The development of drug resistance in human pathogens against commonly used antibiotics and antifungal has necessitated a search for new antimicrobial substances. The antimicrobial activity of ethanol extracts of *Adansonia digitata* fruit pulp, *Hibiscus sabdariffa* flower, *Hyphaene thebaica* fruit pulp and *Ziziphus spina Christi* fruit pulp was determined against the Gram-positive bacterial strains (*B. subtilis* and *S. aureus*), the Gram-negative bacterial strains (*E. coli* and *P. aeruginosa*) and fungal strains (*A. niger* and *C. albicans*) using the disc diffusion method. As shown in Table 1, all plants extracts tested herein revealed antimicrobial activity showing various selectivity for each microorganism. Inhibition zone diameter varied with degree of efficacy and different phyto-constituents of plant on the organism tested. A comparison between the antimicrobial activities of the different plants extracts are graphically represented in Figure 1.

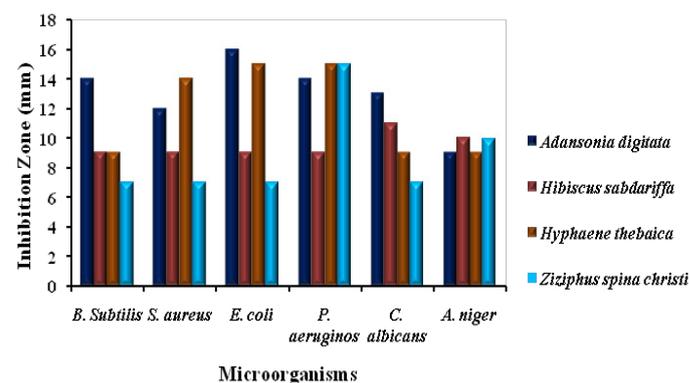


Figure 1: Antimicrobial activity of ethanol extract of selected Sudanese

Table 1: Antimicrobial activity of ethanolic extract of some Sudanese plants

Sample (2.0 mg mL^{-1})	MDIZ (Mean diameter of growth inhibition zone , mm)					
	Bacteria strain					
	<i>B. Subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginos</i>	<i>C. albicans</i>	<i>A. niger</i>
<i>Adansonia digitata</i> (fruit)	14.0 \pm 0.0	12.0 \pm 0.0	16.0 \pm 0.0	14.0 \pm 1.4	13.0 \pm 0.0	9.0 \pm 0.0
<i>Hibiscus sabdariffa</i> (flower)	9.0 \pm 0.7	9.0 \pm 1.4	9.0 \pm 0.0	9.0 \pm 0.7	11.0 \pm 0.0	10.0 \pm 0.7
<i>Hyphaene thebaica</i> (fruit)	9.0 \pm 0.0	14.0 \pm 0.0	15.0 \pm 0.7	15.0 \pm 0.0	9.0 \pm 0.0	9.0 \pm 0.0
<i>Ziziphus spina christi</i> (fruit)	7.0 \pm 0.0	7.0 \pm 0.0	7.0 \pm 0.0	15.0 \pm 0.0	7.0 \pm 0.0	10.0 \pm 0.7

Interpretation of results: MDIZ (mm) : < 9 mm: Inactive ; 9-12 mm: Partially active; 13-18mm: Active ; >18 mm: Very active .

medicinal plants

All plant extracts showed activity against *B. subtilis*, with diameter of inhibition zones were in the range of 7.0-14.0 mm. The highest activity was obtained from fruit pulp of *Adansonia digitata*. This is similar to the findings of Alrasheid *et al.*^[27], who have reported that, ethanolic extract of *Adansonia digitata* fruit pulp showed activity against *B. subtilis* with inhibition zone of 12.0 mm. The Gram-positive *S. aureus* was susceptible in an inhibition zone of 7.0 to 14.0 mm while the Gram-negative *E. coli* was susceptible in an inhibition zone of 7.0 to 16.0 mm. The maximum inhibitions were shown by fruit pulp of *Hyphaene thebaica* extract and fruit pulp extract of *Adansonia digitata* against *S. aureus* and *E. coli*, respectively. These findings are in line with studies by Aboshora *et al.*^[22] and Alrasheid *et al.*^[27], who showed that ethanol extract of *Hyphaene thebaica* fruit pulp *Adansonia digitata* fruit pulp were effective against *S. aureus* and *E. coli*, respectively. The tested extracts showed inhibitory activity against *P. aeruginos* with inhibition zones values between of 9.0 and 15.0 mm and the best antibacterial activity was shown by both extracts of *Hyphaene thebaica* and *Ziziphus spina Christi* fruits pulp. These results were agreeing with that obtained by Taha *et al.*^[28] and Temerk *et al.*^[29], who found that *Hyphaene thebaica* and *Ziziphus spina Christi* fruits pulp showed antibacterial efficacy against *P. aeruginos* with inhibition zone of 11.3 mm and 20 mm, respectively. Both ethanolic extracts of *Adansonia digitata* fruit pulp and *Hyphaene thebaica* fruit pulp exhibited good inhibition effects against Gram-positive and Gram-negative bacteria. This may be due to the presence of broad-spectrum antibiotic compounds in these extracts. Previous study demonstrated that, alcoholic extract of *Adansonia digitata* fruit displayed the wide spectrum of activity against multi-drug resistant (MDR) bacteria^[30]. The antimicrobial activity of these plants was attributed to their rich content of secondary metabolites^[28].

The ethanol extract of *Adansonia digitata* fruit pulp showed high antifungal activity with inhibition zone (13.0 mm) and low activity in *Ziziphus spina christi* (7.0 mm) against *C. albicans*. Both *Hibiscus sabdariffa* (flower) and *Ziziphus spina christi* (fruit pulp) exhibited higher antifungal effects (inhibition zone of 10.0 mm) than *Adansonia digitata* (fruit pulp) *Hyphaene thebaica* (fruit pulp) (inhibition zone of 9.0 mm) against *A. niger*.

Antioxidant activity

In this study, the antioxidant activity of the *Adansonia digitata*, *Hibiscus sabdariffa*, *Hyphaene thebaica* and *Ziziphus spina christi* ethanol extracts were evaluated using DPPH and ABTS assays by determining the total antioxidant capacity of the extract compared with the reference standard antioxidant ascorbic acid. The antioxidant activity, measured as percentage inhibition

of DPPH and ABTS free radicals scavenging activity.

Samples extracts with reasonable antioxidant activity were further evaluated for their 50% inhibitory concentration (IC₅₀). A lower IC₅₀ value corresponds to a larger scavenging activity, the results are shown in Table 2.

DPPH is a stable free radical compound that has been widely used to evaluate antioxidant activity of various samples in a relatively short time compared with other methods^[1]. A hydrogen-donating antioxidants scavenge DPPH radical and change its color changes from purple to yellow. The radical-scavenging activity can be monitored as a decrease in absorbance of DPPH solution at 517 nm^[31].

As shown in Table 2, The highest radical scavenging activity was showed by the extract of *Adansonia digitata* fruit pulp (77.4%) with IC₅₀ 0.273 µg mL⁻¹, followed by the extract of *Hyphaene thebaica* fruit pulp (31.25%) and *Hibiscus sabdariffa* flower revealed weak DPPH scavenging activity (24.4%). The ethanol extract of *Ziziphus spina christi* fruit pulp has no relevant antioxidant activity in DPPH radical scavenging assay.

The previous study by Alrasheid *et al.*^[27] reported that, ethanolic extract of Sudanese *Adansonia digitata* fruit pulp showed an excellent antioxidant activity (83.98%) when measured using DPPH radical scavenging assay. The result of DPPH scavenging ability of the *Hyphaene thebaica* fruit pulp, *Hibiscus sabdariffa* flower and *Ziziphus spina christi* fruit pulp extracts in the present study is lower than those previously reported^[13, 32, 33]. This differences may be referred to different cultivars area of the plants, type of extracting solvents and method of extraction.

Furthermore, the antioxidants activities of the extracts have been measured by ABTS assay. This assay measures the relative antioxidant ability of extracts to scavenge the radical-cation ABTS⁺, produced by the oxidation of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate^[34]. In the present study it was found that, the highest result of antioxidant activity measured by ABTS method was in *Hyphaene thebaica* fruit pulp (79.02%) with IC₅₀ value (0.0808 µg mL⁻¹) followed by *Adansonia digitata* fruit pulp (72.12%) with IC₅₀ value (0.1326 µg mL⁻¹), *Hibiscus sabdariffa* flower showed (57.37%) with IC₅₀ value (0.4174 µg mL⁻¹) and *Ziziphus spina christi* fruit pulp showed moderate antioxidant activity by ABTS method (43.50%). It was observed that, all values of antioxidant activity of plants ethanolic extracts obtained by ABTS assay are relatively higher than those obtained by DPPH assay except *Adansonia digitata* fruit pulp extract which has been shown greater activity in DPPH assay. The results of current study indicated that the ethanolic extracts of fruit of *Adansonia digitata* and *Hyphaene thebaica* fruits pulp have a noticeable effect on scavenging free radicals. This may be related to the concentration of their phenolic compounds. The extracts of *Adansonia digitata* and *Hyphaene thebaica* fruits extracts may be valuable source of bioactive compounds and

Table 2: Antioxidant activity of plants extract by DPPH and ABTS methods

Sample	DPPH %	IC ₅₀ (µg mL ⁻¹)	ABTS %	IC ₅₀ (µg mL ⁻¹)
<i>Adansonia digitata</i> (fruit)	77.40±0.20	0.273	72.12 ±0.02	0.1326
<i>Hibiscus sabdariffa</i> (flower)	24.40 ±0.00	-	57.37 ±0.00	0.4174
<i>Hyphaene thebaica</i> (fruit)	31.25 ±0.00	-	79.02 ± 0.01	0.0808
<i>Ziziphus spina Christi</i> (fruit)	Inactive	-	43.50 ± 0.15	-
Ascorbic acid(standard)	93.5 ±0.00	-	95.05 ±0.00	-

their fruits could play an important nutritional role in the diet. The related studies showed results indicated that the *Adansonia digitata* and *Hyphaene thebaica* fruits pulp extract could be an important dietary source of phenolic compounds with high antioxidant and anticancer activities^[32,35].

HPLC analysis

Ascorbic acid, Gallic acid and quercetin are three important compounds present in many medicinal plants. They show various biological activities such as antioxidant, antimicrobial and anticancer activity^[12,36].

In the present study, ascorbic acid, gallic acid and quercetin have been determined using HPLC-PDA. Photodiode array detector (PDA) was used for determination lambda max (λ_{max}) of these compounds. It was found that, the λ_{max} of ascorbic acid, gallic acid and quercetin was 245, 272 and 370 nm, respectively. As shown in Figure 2, the retention time of ascorbic acid was 3.5 min, gallic acid was 4.5 min and quercetin was 11.0 min. In order to establish the relationship between peak area and concentration, linear regression analysis was carried out for the investigated standards listed in Table 3. The calibration curves were constructed by plotting peak area processed at λ_{max} of each analyte against the corresponding concentration. Good linearities were obtained for all tested analytes with correlation coefficient exceed 0.9998 over the concentration range studied. It was found that, the calibration curve of ascorbic acid is linear in concentration range (3.0-100 mg L⁻¹), and in concentration range of (0.6-100 mg L⁻¹) for gallic acid and quercetin. The limit of detection (LOD) was established using $LOD = 3.3 \times (s/S)$ and the LOQ = $10 \times (s/S)$, where s is the standard deviation of the intercept and S is the slope of the curve^[37]. As presented in Table 3, good sensitivities have been obtained with LODs and LOQs ranged from 0.096-0.838 mg L⁻¹ and 0.288-2.514 mg L⁻¹, respectively. The concentrations of tested compounds in the samples have been calculated from the established calibration curves equations. The amount of each compound was expressed as mg/100 g dried plants extracts. As shown in Table 4, the ascorbic acid, gallic acid and quercetin were detected in the ethanolic extracts of the all tested plants. It was found that, *Adansonia digitata* fruit pulp extract contains the highest concentration of ascorbic acid (302.11 mg/100 g) and *Ziziphus spina Christi* fruit pulp extract contains the lowest concentration (209.47mg/100 g). This

result is consistent with the fact that *Adansonia digitata* fruit pulp represents the most important natural sources of ascorbic acid and considered a rich source containing levels of vitamin C in comparison to the fruits that are generally considered the best source of ascorbic acid^[35]. Due to its relatively high concentration, ascorbic acid may contribute significantly to the antioxidant activity of *Adansonia digitata* fruit. The highest concentration of gallic acid was detected in flower of *Hibiscus sabdariffa* flower extract (135.60 mg/100 g) and the lowest concentration was detected in *Ziziphus spina Christi* fruit pulp extract (50.64 mg/100 g). No data has been found in the literature about concentration of gallic acid in flower of *Hibiscus sabdariffa*. Fruit of *Hyphaene thebaica* extract contains the highest concentration of quercetin (55.24mg/100 g) and *Adansonia digitata* fruit pulp extract contains the lowest concentration of quercetin (23.44 mg/100 g). The content of quercetin in *Hyphaene thebaica* (fruit pulp) extract obtained in the current study is higher than the one reported by Aamer et al^[38], that found that the water extract of *Hyphaene thebaica* fruit pulp was 0.084 mg/100 g. This difference is maybe due to use ethanol as extracting solvent in present study, which it has been reported as efficient extractor for phenolic compounds^[39]. The reported amounts in literature of ascorbic acid, gallic acid and quercetin in *Adansonia digitata* fruit pulp, *Hibiscus sabdariffa* flower, *Hyphaene thebaica* fruit pulp and *Ziziphus spina christi* fruit pulp are summarized in Table 5. It is difficult to compare our results with those of other authors due to different environmental and geographical origin of analyzed samples.

Table 3: Rang, equations of calibration curves, regression coefficient, limit of detection (LOD) and limit of quantification (LOQ) for ascorbic acid, gallic acid and quercetin

Standard ID	Range (ppm)	Equation	Regression coefficient (r ²)	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)
Ascorbic acid	3.0-100	Y = 39426 X + 8761.8	0.9998	0.838	2.514
Gallic acid	0.6-100	Y = 68660 X + 5657.5	0.9999	0.096	0.288
Quercetin	0.6-100	Y = 70814 X + 43837	0.9999	0.121	0.364

Table 4: Content of ascorbic acid, gallic acid and quercetin in the selected Sudanese plants

Sample ID	Concentration (mg/100 g extract)		
	Ascorbic acid	Gallic acid	Quercetin
<i>Hibiscus sabdariffa</i> (flower)	257.60±0.590	135.60±0.210	51.28±0.0180
<i>Adansonia digitata</i> (fruit)	302.11±0.260	83.01±0.04	23.44±0.002
<i>Hyphaene thebaica</i> (fruit)	225.21±0.064	120.56±0.020	55.24±0.030
<i>Ziziphus spina Christi</i> (fruit)	209.47±0.160	50.64±0.025	29.56±0.030

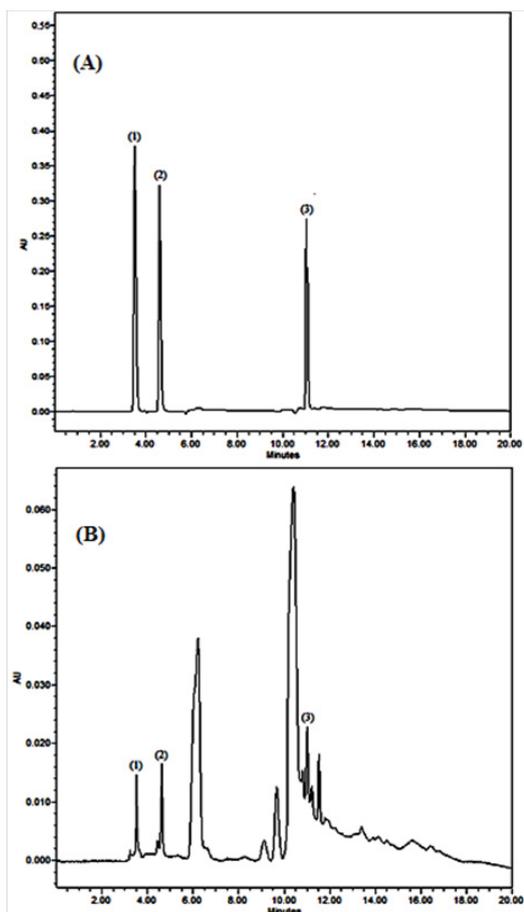


Figure 2: Typical HPLC chromatograms of (A) mixed standards (B) *Hibiscus sabdariffa* flower extract where (1) is Ascorbic acid, (2) Gallic acid, (3) Quercetin.

Conclusion

The antimicrobial and antioxidant activities of ethanolic extracts of some Sudanese plants namely; *Adansonia digitata* fruit pulp, *Hibiscus sabdariffa* flower, *Hyphaene thebaica* fruit pulp and *Ziziphus spina Christi* fruit pulp were investigated and presented. Qualitative and quantitative analysis of ascorbic acid, gallic acid and quercetin of the plants ethanolic extracts were performed using HPLC-PDA. All plants extracts tested herein showed various degrees of antimicrobial activity against the different tested pathogenic strains. The results show that the four selected plants had significant antioxidant activity. The results for antioxidant activities measured using ABTS assay are relatively higher than those measured by DPPH assay except of *Adansonia digitata* fruit pulp extract. HPLC technique has been used for qualitative and quantitative analysis of ascorbic acid, gallic acid and quercetin in the tested samples extract. These three bioactive compounds have been detected in all samples extracts in variable amounts. The findings of this study support the traditional use of the studied Sudanese plants in the treatment of some diseases and they could be promising potential sources of antioxidants and antimicrobial compounds.

References

1. Mohamed Amal, A., Khalil, A.A., El-Beltagi Hossam,

E.S. et al. Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). (2010) GRASAS Y ACEITES 61(1): 67-75.

[Pubmed](#) | [Crossref](#) | [Others](#)

2. Meena Mahesh Chand Meena RK, Patni Vidya Effect of elicitor on quercetin production in cell cultures of *Citrullus colocynthis* (Linn.) Schrad. (2014) Pharma Innovation 3(2): 18-23.

[Pubmed](#) | [Crossref](#) | [Others](#)

3. Salih, N.K., Yahia, E.M. Nutritional value and antioxidant properties of four wild fruits commonly consumed in Sudan. (2015) Int Food Res J 22(6): 2389-2395.

[Pubmed](#) | [Crossref](#) | [Others](#)

4. Elhassan Eiman Hassan, A.R., Ahmmed, E.M., Sirag, N. Standardization of Roselle (*Hibiscus sabdariffa* L.) Calyx cultivated in Sudan. (2014) J Medicinal Plants Res 8(4): 217-222.

[Pubmed](#) | [Crossref](#) | [Others](#)

5. Dah-Nouvlessounon, D., Adjanooun, A., Sina, H., et al. Nutritional and Anti-Nutrient Composition of Three Kola Nuts (*Cola nitida*, *Cola acuminata* and *Garcinia kola*) produced in Benin. (2015) Food Nutr Sci 6: 1395-1407.

[Pubmed](#) | [Crossref](#) | [Others](#)

6. Abdallah, E.M., Elsharkawy, E.R., Ed-dra, A. Biological activities of methanolic leaf extract of *Ziziphus mauritiana*. (2016) Biosci Biotech Res Comm 9(605-614): 605-614.

[Pubmed](#) | [Crossref](#) | [Others](#)

7. Sagwan, S., Rao, D.V., Sharma, R.A. Phytochemical evaluation and quantification of primary metabolites of *Maytenus emarginata* (Willd.) Ding Hou. (2010) J Chemical Pharm Res 2(6): 46-50.

[Pubmed](#) | [Crossref](#) | [Others](#)

8. Ibrahim, M.H., Jaafar, H.Z.E., Ehsan, K., et al. Primary, Secondary Metabolites, Photosynthetic Capacity and Antioxidant Activity of the Malaysian Herb *Kacip Fatimah* (*Labisia pumila* Benth) Exposed to Potassium Fertilization under Greenhouse Conditions. (2012) Int J Mol Sci 13(11): 15321-15342.

[Pubmed](#) | [Crossref](#) | [Others](#)

9. Ruby, T. Plant secondary metabolites: a review. (2015) Int J Engg Res General Sci 3(5): 661-670.

[Pubmed](#) | [Crossref](#) | [Others](#)

10. Gupta, M., Saumyakanti, S., Sohini, M., et al. HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. (2012) Int J Pharmacognosy and Phytochem Res 4(3): 162-167.

[Pubmed](#) | [Crossref](#) | [Others](#)

11. Bénédicte, L., Laurent, P., Teissedre, P.L.. Evolution of Analysis of Polyphenols from Grapes, Wines, and Extracts. (2013) Molecules 18(1): 1076-100.

[Pubmed](#) | [Crossref](#) | [Others](#)

12. Verma, N., Trehan, N. HPLC Analysis of Methanolic Extract of Herbs for Quercetin Content. (2013) J Pharmacognosy and Phytochemistry 2(1): 159-162.

[Pubmed](#) | [Crossref](#) | [Others](#)

13. Obouayeba, A.P., Bernard, N., Diabate, S., et al. Phytochemical and Antioxidant Activity of Roselle (*Hibiscus Sabdariffa* L.) Petal Extracts. (2014) Res J Pharmaceutical,

- Biol Chem Sci 5(2): 1453-1465.
[Pubmed](#) | [Crossref](#) | [Others](#)
14. Middha, S.K., Usha, T., Pande, V. HPLC Evaluation of Phenolic Profile, Nutritive Content, and Antioxidant Capacity of Extracts Obtained from Punica granatum Fruit Peel. (2013) Evidence-Based Complementary and Alternative Medicine 1-6.
[Pubmed](#) | [Crossref](#) | [Others](#)
 15. Hegde P. Agrawal, P., Gupta, P.K. Polyphenols- a useful biomaterial: A Review. (2016) J Environ Res Development 10(3): 547-554.
[Pubmed](#) | [Crossref](#) | [Others](#)
 16. Mitić, S., Kostić, D.A., Nasković-Đokić D.C., et al. Rapid and Reliable HPLC Method for the Determination of Vitamin C in Pharmaceutical Samples. (2011) Tropical J Pharm Res 10(1): 105-111.
[Pubmed](#) | [Crossref](#) | [Others](#)
 17. Entessar, H.A., Mosawe., Al., Iman, I., et al. The Extraction and Purification of Gallic Acid from the Pomegranate Rind. (2012) Al- Mustansiriyah J Sci 23(6): 53-60.
[Pubmed](#) | [Crossref](#) | [Others](#)
 18. Vijaya Sri, K., Vijaya Ratna, J., Annapurna A., et al. Reversed-Phase HPLC Method for Determination of Quercetin in Human Plasma. (2009) Asian J Chem 21(1): 101-104.
[Pubmed](#) | [Crossref](#) | [Others](#)
 19. Sanghavi, N., Bhosale, S., Malode, Y. RP-HPLC method development and validation of Quercetin isolated from the plant Tridax procumbens L. (2014) J Scientific Innovative Res 3(6): 594-597.
[Pubmed](#) | [Crossref](#) | [Others](#)
 20. Alves M.J. Ferreira, I.C.F.R., Froufe H.J.C., et al. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. (2013) J Appl Microbiol 115(2):1-12.
[Pubmed](#) | [Crossref](#) | [Others](#)
 21. Akinwunmi, A.O., Olanlokun, J.O., Habib, T., et al. Molecular docking analysis of apigenin and quercetin from ethylacetate fraction of Adansonia digitata with malaria-associated calcium transport protein: An in silico approach. (2019) Heliyon 5(e02248): 1-9.
[Pubmed](#) | [Crossref](#) | [Others](#)
 22. Aboshora, W., Lianfu, Z., Dahir, M., et al. Effect of Extraction Method and Solvent Power on Polyphenol and Flavonoid Levels in Hyphaene Thebaica L Mart (Arecaceae) (Doum) Fruit, and its Antioxidant and Antibacterial Activities. (2014) Tropical J Pharm Res 13(12): 2057-2063.
[Pubmed](#) | [Crossref](#) | [Others](#)
 23. Brito, S.M.O., Coutinho, H.D.M., Talvani, A., et al. Analysis of bioactivities and chemical composition of Ziziphus joazeiro Mart. using HPLC-DAD. (2015) Food Chem 186: 185-191.
[Pubmed](#) | [Crossref](#) | [Others](#)
 24. Kil, H.Y., Seong, E.S., Ghimire, B.K., et al. Antioxidant and antimicrobial activities of crude sorghum extract. (2009) Food Chem 115(4): 1234-1239.
[Pubmed](#) | [Crossref](#) | [Others](#)
 25. Shimada, K., Fujikawa, K., Yahara, K., et al. Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. (1992) J Agric Food Chem 40: 945-948.
[Pubmed](#) | [Crossref](#) | [Others](#)
 26. Re, R., Pellegrini, N., Proteggente, A., et al. Antioxidant activity applying an improved ABTS radical decolorization assay. (1999) Free Radical Biol Med 26: 1231-1237.
[Pubmed](#) | [Crossref](#) | [Others](#)
 27. Alrasheid Ayat Ahmed, A.S.A., EltilibSahar, H., Alnour Mawa, I., et al. The effect of blending of extracts of Sudanese Adansonia digitata and Tamarindus indica on their antioxidant, anti-inflammatory and antimicrobial activities. (2019) J Pharmacognosy and Phytotherapy 11(2): 28-34.
[Pubmed](#) | [Crossref](#) | [Others](#)
 28. Taha Ghada, A., Elgebaly Hassan, A., Mahalel Usama, A., et al. Metabolomic Profiling and Antioxidant, Anticancer and Antimicrobial Activities of Hyphaene thebaica. (2020) Processes 8(3): 1-13.
[Pubmed](#) | [Crossref](#) | [Others](#)
 29. Temerk, H.A. Sayed W.F., Hassan F.S. Antibacterial Effect of Phytochemical Extracts from Ziziphus-spina christi against Some Pathogenic Bacteria. (2017) Egyptian J Botany 57(3): 595 - 604.
[Pubmed](#) | [Crossref](#) | [Others](#)
 30. Djeussi Doriane, E., Seukep Jackson, A., FankamAimé, G., et al. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. (2013) BMC Complement Altern Med 164:1-8.
[Pubmed](#) | [Crossref](#) | [Others](#)
 31. Azizi Ali, P.M. Regional variations of antioxidant capacity and phenolic properties in the Iranian jujube collection. (2016) J Herbal Drugs 6(4):199-209.
[Pubmed](#) | [Crossref](#) | [Others](#)
 32. Abou -Elalla, F.M. Antioxidant and anticancer activities of doum fruit extract (Hyphaene thebaica). (2009) African J Pure Applied Chem 3(10):197-201.
[Pubmed](#) | [Crossref](#) | [Others](#)
 33. Alhakmani, F., A.K.S., Ahmad Aftab. Determination of total phenol, in-vitro antioxidant and anti-inflammatory activity of seeds and fruits of Zizyphus spina-christi grown in Oman. (2014) Asian Pacific Journal of Tropical Biomedicine 4(Suppl 2): S656-S660.
[Pubmed](#) | [Crossref](#) | [Others](#)
 34. Al-Rimawi, F., Abu-Lafi, S., Abbadi, J., et al. Analysis of phenolic and flavonoids of wild ephedra alata plant extracts by LC/PDA and LC/MS and their antioxidant activity. (2017) Afr J Tradit Complement Altern Med 14(2):130-141.
[Pubmed](#) | [Crossref](#) | [Others](#)
 35. Vertuani, S., Broccioli, E., Valentina, B., et al. Antioxidant capacity of Adansonia digitata fruit pulp and leaves. (2002) Acta Phytotherapeutica 2(2): 1-7.
[Pubmed](#) | [Crossref](#) | [Others](#)
 36. Aleksandra, O., OMA, Gudej, J. Quantitative determination of ellagic acid and gallic acid in Gum rivalel L. and G. urbanum L. (2014) Acta Biologica Cracoviensia Series Botanica 56(2):74-78.
[Pubmed](#) | [Crossref](#) | [Others](#)
 37. Fernandes Jos'e, O. SC. Application of matrix solid-phase dispersion in the determination of acrylamide in potato chips. (2007) J Chromatogr A 1175(1):1-6.
[Pubmed](#) | [Crossref](#) | [Others](#)

38. Aamer, A.R. Characteristics of aqueous doum fruit extract and its utilization in some novel products. (2016) Ann Agriculture Sci 61(1):25–33.
[Pubmed](#) | [Crossref](#) | [Others](#)
39. Mônica, C.P., Edira, C.B.A. Effect of different extracting solvents on antioxidant activity and phenolic compounds of a fruit and vegetable residue flour. (2016) Scientia Agropecuaria 7(1): 07 – 14.
[Pubmed](#) | [Crossref](#) | [Others](#)
40. Inês Da-Costa-Rocha, Bonnlaender, B., Sievers, H., et al. Hibiscus sabdariffa L. – A phytochemical and pharmacological review. (2014) Food Chem 165: 424–443.
[Pubmed](#) | [Crossref](#) | [Others](#)
41. A Abdel-Rahman Nawal AII, Elshafe'a ELrakha BB. Characterization of some Sudanese Edible Forest Fruits. (2014) J Agri-Food App Sci 2(2): 39-44.
[Pubmed](#) | [Crossref](#) | [Others](#)
42. Abdalla Ashraf Mohamed Ahmed, EHBG. Variation of Physicochemical Characteristics of Ziziphus spina-christi Fruits from Three Geographical Sites in Sudan. (2014) Proceeding of the 5th Annual Conference of Postgraduate Studies and Scientific Research.
[Pubmed](#) | [Crossref](#) | [Others](#)
43. Tembo, D.T., Holmes, M.J., Marshall, L.J. Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (*Adansonia digitata*) pulp from Malawi. (2017) J Food Composition Anal 58: 40-51.
[Pubmed](#) | [Crossref](#) | [Others](#)
44. Mohammed Ghena M ZNS. Comparison between The Chemical And Antioxidant Content of The Egyptian And Saudi Doum Fruit. (2018) Int J Pharm Res Allied Sci 7(1): 87-92.
[Pubmed](#) | [Crossref](#) | [Others](#)
45. Basuny Amany, M., Arafat, S.A., Farag, H.A. Utilization from fruits and leaves of napek (*Ziziphus spina-christi* L.) as a source of bioactive components. (2013) J Biotechnol 4(7):16-28.
[Pubmed](#) | [Crossref](#) | [Others](#)
46. Hossain, H., Nayeeb, A.P., Rahman, S.E., et al. HPLC Profiling and Antioxidant Properties of the Ethanol Extract of Hibiscus tiliaceus Leaf Available in Bangladesh. (2015) Eur J Med Plants 7(1): 7-15.
[Pubmed](#) | [Crossref](#) | [Others](#)