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Antimicrobial, Antiparasitic and Antioxidant Activities of Medicinal Plants from Sudan

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Abstract

The rural population of Sudan has traditionally used medicinal plants for treatment of several ailments and microbial infections. In the present study, we have investigated the antibacterial, antitrypanosomal, antiplasmodial, and antioxidant properties of selected Sudanese medicinal plants using various *in vitro* assays. Methanolic extracts of various parts of the plants were tested against six bacterial strains *(Bacillus subtilis, Bacillus aquimaris, Clavibacter michiganensis, Escherichia coli, Erwinia amylovora, and Pseudomonas syringae)* using agar diffusion and minimal inhibitory concentration (MIC) methods. The anti plasmodial activity was tested against a chloroquine sensitive strain of *Plasmodium falciparum* NF54, whereas the antitrypanosomal activity was evaluated against *Trypanosoma brucei rhodesiense* STI900 (African strain). The antioxidant activity of the plant extracts was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging. Various extract showed antibacterial, antiparasitic and antioxidant activities. *Acacia nilotica, Ocimum basilicam, Ziziphus spina-christi, Balanites aegyptiaca, Sonchus oleraceus, Punica granatum, Mimosa pigra* and *Ixora coccinea* were the most interesting ones with antioxidative, antiplasmodial, antitrypanosomal and antibacterial activities. By means of preparative HPLC, HPLC-ESI-TOF, HPLC-ESI-MS^n, ^{1}H-NMR and ^{13}C-NMR, thirteen phyto constituents were isolated and identified in the methanolic extracts of *Z. spina-christi*, *S. oleraceus*, and *H. sabdariffa* including chlorogenic acids, flavonoid glycosides, coumarins and derivatives. Sudanese plants represent an important alternative source of natural antioxidants, antiparasitic and antimicrobials. To our knowledge, this is the first report about the antibacterial, antiparasitic and antioxidant activities of some of these plants.

Keywords: Medicinal plants; Antiplasmodial; Antitrypanosomal; Antibacterial; Antioxidant; Preparative-HPLC

Introduction

Medicinal plants continue to play a vital role as therapeutic agents in primary health care in developing countries [1]. Sudan is located in tropical Africa and has high plant diversity and a multinational population. In Sudan and other developing countries, traditional medicine plays a major role particularly in rural regions due to both economic and cultural reasons [2]. Comprehensive ethnobotanical investigations on Sudanese folk medicine was reported previously [3-6]. (The ethnomedicinal data of selected plant materials are provided in (Table S1) in the Supporting information).

The frequent use of medicinal plants for treatment of different diseases has encouraged a number of researchers to study their biological activities [7,8]. Additionally, natural products can contribute to the discovery of novel antimicrobial [9], and antioxidant components [10]. A number of pharmaceutical studies have demonstrated the antibacterial, antimalarial, antitrypanosomal, and antioxidant activities of Sudanese medicinal plants [11,12,2,13-18]. It is well-known that plants, which are rich in a diversity of secondary metabolites such as polyphenols, tannins, terpenoids and alkaloids are usually interesting for their antiparasitic, antimicrobial, and antioxidant activities [19-24]. The antioxidant hypothesis is however under discussion, and recent evidence suggested that its role has heavily been overestimated [25]. Therefore, the aim of the present study was to evaluate the antibacterial, antiplasmodial, antitrypanosomal, and antioxidant activities of some Sudanese medicinal plants. The antimicrobial effect of some phenolic compounds extracted from edible and medicinal plants from Europe was also assessed. We have also isolated thirteen bioactive phenolic constituents from *Z. spina-christi*, *S. oleraceus* and *H. sabdariffa* by preparative-HPLC (PHPLC).

Materials and Methods

Chemicals and standards

All chemicals, solvents and authentic standards used in this study were analytical grade. 5-O-caffeoylquinic acid (>98%), 3-O-caffeoylquinic acid (>98%) and 4-O-caffeoylquinic acid (>96.45%) were purchased from Phytolab (Vestenbergsgreuth, Germany). Quercetin 3-O-(6-O-rhamnosyl-glucoside) (rutin) (≥94%), quercetin 3-O-glucoside (90%), esculin (≥98%), luteolin 7-O-glucoside (≥98%), gallic acid (≥99%), methanol (≥99.9%), methanol-d_4 (99.96%) and DMSO (≥95%) were purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water with a resistance of 18.2M was deionized in a Milli-Q system (Sartorius Stedim Biotech GmbH, Germany).

Plant materials and preparation

All Sudanese plant samples were freshly collected June and July 2010 from their natural habitats in Omdurman (15°38' North, 32° 26' East) and Khartoum (15°33' North, 32°31' East), Sudan. The voucher specimens were identified by Dr. Hayder Abdel Gadir of Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, where the specimens were also deposited. All plant species, part used and voucher specimen numbers are presented in (Table 1). The plants were selected based on previous studies [3-6,17,26-35]. (See also Table S1 in the Supporting information).

The flowers of *Catharanthus roseus* were purchased from Wassenaar Living Garden (Bremen, Germany). Quince fruits (*Cydonia oblonga*), green coffee (*Coffea arabica*) beans and black tea (*Camellia sinensis*) leaves were purchased from local markets in Bremen, Germany, while the cocoa (*Theobroma cacao*) beans and *Bobgunnia madagascariensis* pods were donated by Barry Callebaut, Wieze, Belgium and Prof. Dr. Philip Stevenson, Royal Botanic Gardens, Kew, Richmond, Surrey, UK, respectively.

The sample preparation was achieved as described in our previous work [36] (Table 1).

Evaluation of antibacterial activities

Bacterial susceptibility determinations: The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation. The MIC was determined by a twofold dilution assay in Mueller-Hinton broth (MHB) (Becton Dickinson, Heidelberg, Germany). Three Gram-positive bacterial strains (*Bacillus subtilis* S168, *Bacillus aquimaris* MB-2011, and *Clavibacter michiganensis* GSPB 390 and three Gram-negative bacterial strains (*Escherichia coli* DH5α, *Erwinia amylovora* 1189, and *Pseudomonas syringae* pv tomato DC300) were selected as model organisms to evaluate the antibacterial activity of the crude plant extracts and phenolic compounds. The plates were incubated overnight at 28 °C except for *E. coli*, for which incubation was done at 37 °C. All tests were done in triplicate following the National Center for Clinical Laboratory Standards recommendations (National Committee for Clinical Laboratory Standards (NCCLS), 2000).

Agar diffusion assays: Agar diffusion assays were performed as in our previous study [37].

Evaluation of antiplasmodial and antitrypanosomal activities

The antiplasmodial and antitrypanosomal activities were evaluated *in vitro* against a chloroquine sensitive strain of *Plasmodium falciparum* NF54 and *Trypanosoma brucei rhodesiense* STI900 (African strain), respectively. Both assays were carried out at two different concentrations (2 and 10 µg/mL). These experiments were conducted in collaboration with Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland as previously described [38].

Evaluation of antioxidant activities

The scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals of the crude plant extracts was measured according to a method previously described [39]. Assays were carried out in 3mL reaction mixtures containing 2mL of 0.1mM DPPH-ethanol solution, 0.9ml of 50mM Tris-HCl buffer (pH 7.4) and 0.mL of plant extracts at two different concentrations (10 and 100µg/mL). The reaction mixture was vortexed and left in the dark at room temperature (27 °C) for 30min. The absorbance was measured spectrophotometrically at 517nm. Gallic acid was used as a positive control, while ethanol was used as a blank sample. The inhibitory effect of DPPH was calculated using the standard equation [40]. The experiments were conducted in triplicates, and the data are given as mean values ± standard deviation (SD).

HPLC & HPLC-MS^n

The HPLC separation and HPLC-MSn analysis was achieved as previously described [41,42].

Preparative-HPLC isolation

Preparative-HPLC isolation of compound 1-13 was carried out as in our previous study [37].

NMR

^{1}H NMR and ^{13}C NMR spectra were acquired on a JEOL ECX-400 spectrometer operating at 400MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in CD_3OD using a 5mm probe. The chemical shifts (δ) are reported in parts per million (ppm) and were referenced to the residual solvent peak. The coupling constants (J) are quoted in hertz (Hz).

Esculin (5): ^{1}H-NMR (400 MHz, CD_3OD) δ_H 6.28 (d, J 9.62 Hz, H-3), 7.82 (d, J 9.16 Hz, H-4), 7.18 (s, H-5), 7.0 (s, H-8), 4.96 (d, J 7.33 Hz, H1'), 3.54 (dd, J 9.27 and 7.3 Hz, H2'), 3.50 (dd, J 9.2 and 7.3 Hz, H-3'), 3.40 (dd, J 9.62 and 8.7 Hz, H-4'), 3.51 (m, H-5'), 3.92 (dd, J 12.36 and 2.29 Hz, H6'a), 3.70 (dd, J 11.91 and 5.5 Hz, H6'b); ^{13}C-NMR (100 MHz, CD3OD) δ_C 61.02 (C-6'), 69.93 (C-4'), 73.36 (C-2'), 76.03 (C-3'), 77.16 (C-5'), 101.55 (C-1'), 104.18 (C-8), 112.49 (C-3), 113.25 (C-10), 114.04 (C-5), 144.40 (C-6), 145.10 (C-4), 147.71 (C-7), 149.52 (C-9), 162.29 (C-2). These data were in agreement with those reported in the literature [43,44].

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Luteolin 7-O-glucoside (6): ^{1}H-NMR (400 MHz, CD3OD) δ_H 6.28 (1H, d, J 7.0 Hz, H-1''), 3.35 – 3.55 (4H, m, H-2'',3'',4'',5''), 3.91 (1H, dd, J 11.91 and 1.83 Hz, H-6''a), 3.70 (1H, dd, J 11.91 and 5.5 Hz, H-6''b), 6.59 (1H, s, H-3), 6.78 (1H, d, J 2.29 Hz, H-6), 6.48 (1H, d, J 2.29 Hz, H-8), 7.38 (1H, d, J 1.83 Hz, H-2'), 6.87 (1H, d, J 8.70 Hz, H-5'), 7.40 (1H, dd, J 8.24 and 2.29 Hz, H-6'); ^{13}C-NMR (100 MHz, CD3OD) δC 61.11 (C-6''), 69.93 (C-4''), 73.39 (C-2''), 76.52 (C-3''), 77.05 (C-5'), 102.61 (C-1''), 165.55 (C-2), 102.61 (C-3), 182.69 (C-4), 161.55 (C-5), 99.79 (C-6), 163.66 (C-7), 94.70 (C-8), 157.50 (C-9), 105.73 (C-10), 122.20 (C-1'), 112.70 (C-2'), 146.02(C-3'), 145.21 (C-4'), 115.53 (C-5'), 119.14 (C-6') (45,46)

Results and Discussion

Phytochemical profiling and characterization of the isolated constituents

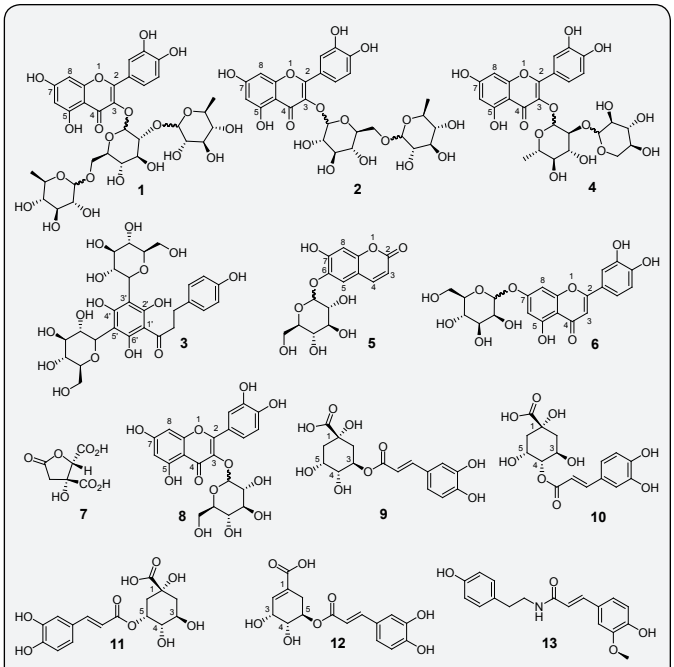

Figure 1: Chemical structures of compounds 1-13 isolated from *Z. spina-christi*, *S. oleraceus* and *H. sabdariffa*.

A detailed phytochemical analysis using HPLC-MS was carried out on *Z. spina-christi*, *S. oleraceus* and *H. sabdariffa* in previous work [47-50] From these plants in our present study, preparative HPLC was used to isolate four compounds (1-4) from the methanolic extracts of *Z. spina-christi*, two compounds (5 and 6) from S.oleraceus and seven compounds (7-13) from *H. sabdariffa*. The structures of the isolated compounds (Figure 1) were elucidated by HRMS, tandem MS, UV chromatograms, retention times (RT), authentic standards and data obtained from literature (Figure 2 & Table 2). Chromatographic resolution and MS data were considered for isolated compounds showing a poor NMR spectral resolution. Tandem MS data and preparative HPLC chromatograms are provided in the supporting information. With these agreements compounds (1-13) were identified as quercetin 3-O-(2,6-di-O-rhamnosyl-glucoside), quercetin 3-O-(6-O-rhamnosyl-glucoside) (rutin), phloretin 3',5' di-C-glucoside, quercetin 3-O-(2-O-rhamnosyl-pentoside), esculin, luteolin 7-O-glucoside, hibiscus acid, quercetin 3-O-glucoside, 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 5-O-caffeoylshikimic acid and N-feruloyltyramine, respectively. The purity of these compounds (Table 1) was determined as in our previous study [37] by total ion chromatograms in negative ion mode and UV chromatograms at 280nm. (Table 2) Retention times, high resolution MS data and amounts of the isolated constituents.

Table 1: List of the plant investigated in this study.

Family	Plant Species	Part Used	Voucher
Malvaceae	*Hibiscus sabdariffa L.*	Petals	Hi-sa-09
Fabaceae	*Acacia nilotica (L.) Willd. ex Del.*	Fruits	Ac-ni-10
Bombacaceae	*Adansonia digitata L.*	Fruits	Ad-di-11
Rhamnaceae	*Ziziphus spina-christi (L.) Desf.*	Leaves	Zi-sp-ch-04
Rhamnaceae	*Ziziphus spina-christi (L.) Desf.*	Fruits	Zi-sp-ch-04
Balanitaceae	*Balanites aegyptiaca (L.) Del*	Bark	Ba-ae-03
Balanitaceae	*Balanites aegyptiaca (L.) Del*	stem	Ba-ae-03
Balanitaceae	*Balanites aegyptiaca (L.) Del*	Leaves	Ba-ae-03
Asteraceae	*Sonchus oleraceus L.*	Whole plant	So-ol-01
Asteraceae	*Ambrosia maritima L.*	Whole plant	Am-ma-02
Rubiaceae	*Ixora coccinea L.*	Stem	Ix-co-07
Rubiaceae	*Ixora coccinea L.*	Leaves	Ix-co-07
Lamiaceae	*Ocimum basilicam L.*	Aerial part	Oc-ba-05
Fabaceae	*Mimosa pigra L.*	Stem	Mi-pi-06
Fabaceae	*Mimosa pigra L.*	Leaves	Mi-pi-06
Annonaceae	*Annona senegalensis Pers.*	Stem	An-se-08
Annonaceae	*Annona senegalensis Pers.*	Leaves	An-se-08
Tiliaceae	*Grewia tenax (Forssk.) Fiori.*	Fruits	Gr-te-12
Cucubitaceae	*Cucurbita moschata Duch.*	Seeds	Cu-mo-13
Apocynaceae	*Catharanthus roseus L.*	Leaves	-
Leguminosae	*Bobgunnia madagascariensis (Desv.) J. H. Kirkbr. & Wiersema*	Pods	STVP-1017
Rosaceae	*Cydonia oblonga Miller. (quince)*	Fruits	-
Lythraceae	*Punica granatum L. (pomegranate)*	Peels	
Lythraceae	*Punica granatum L.(pomegranate)*	Juice	-
Rubiaceae	*Coffea arabica L. (green coffee)*	Beans	-
Malvaceae	*Theobroma cacao L. (cocoa)*	Beans	-
Theaceae	*Camellia sinensis L. (black tea)*	Leaves	-

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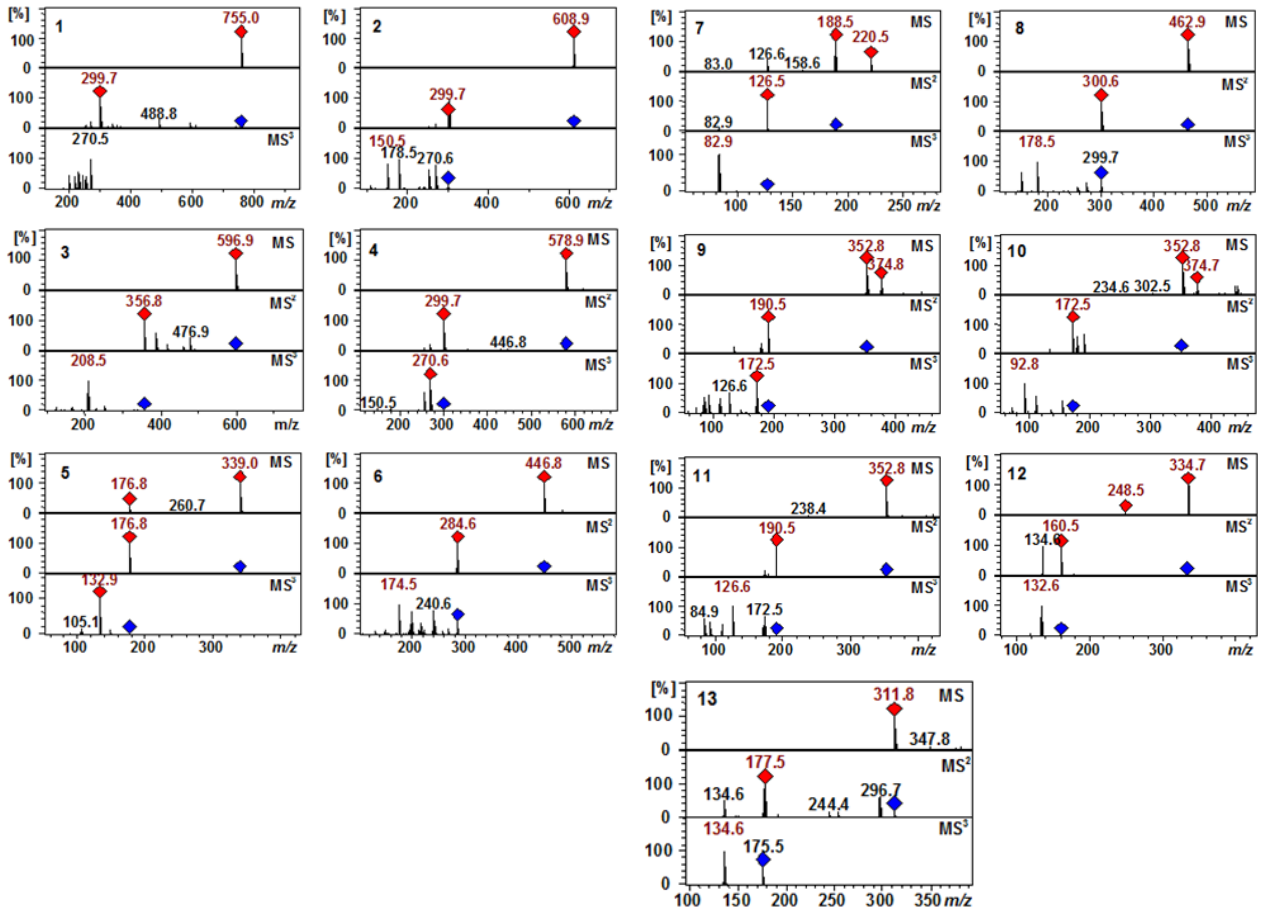

Figure 2: Tandem MS spectra of compounds 1-13 in negative ion mode.

Table 2: Retention times, high resolution MS data and amounts of the isolated constituent.

No.	Compound identity	RT (min)	Mol. formula	Ther. m/z [M−H]	Exp. m/z [M−H]	Err. [ppm]	Amount [mg/5g]	Purity [%]	Ref./ Std.
1.	Quercetin 3-O-(2,6-di-O-rhamnosyl-glucoside)	15.6	C33H40O20	755.204	755.2055	-2	2.5	100	[68]
2.	Quercetin 3-O-(6-O-rhamnosyl-glucoside) (rutin)	41	C27H30O16	609.1461	609.1478	-2.8	25	85.62	Std.
3.	Phloretin 3',5' di-C-glucoside	36.8	C27H34O15	597.1825	597.185	-4.2	9	55.47	[49]
4.	Quercetin 3-O-(2-O-rhamnosyl-pentoside)	46.2	C26H28O15	579.1355	579.1351	0.8	2.6	74.95	[49]
5.	Esculin	15	C15H16O9	339.0722	339.0726	-1.3	1.1	100	Std.
6.	Luteolin 7-O-glucoside	36.6	C21H20O11	447.0933	447.0929	0.8	0.9	100	Std.
7.	Hibiscus acid	4	C6H6O7	189.0041	189.0039	0.9	54.1	100	[67]
8.	Quercetin 3-O-glucoside	41.7	C21H20O12	463.0882	463.0877	1.1	2.8	100	Std.
9.	3-O-caffeoylquinic acid	13.6	C16H18O9	353.0878	353.0878	0.1	11.1	90.61	Std.
10.	4-O-caffeoylquinic acid	21.2	C16H18O9	353.0878	353.0877	0.3	12.1	100	Std.
11.	5-O-caffeoylquinic acid	20.9	C16H18O9	353.0878	353.0881	0.8	6	84.72	Std.
12.	5-O-caffeoylshikimic acid	27	C16H16O8	335.0772	335.077	0.8	0.6	79.62	[41]
13.	N-feruloyltyramine	42.7	C18H19O4	312.1241	312.1241	0.2	0.7	78.42	[67]

Evaluation of antibacterial activities

The antibacterial activities of the crude plant extracts and some selected phenolic compounds isolated by preparative HPLC from the plant material analysed in this study were evaluated for their efficacies against Gram-positive and Gram-negative bacteria. As suitable model organisms *B. subtilis* S168, *B. aquimaris* MB-2011, and *C. michiganensis* GSPB 390 were chosen for Gram-

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positive and *E. coli* DH5α, *E. amylovora* 1189, and *P. syringae pv tomato* DC300 for Gram-negative. The organisms chosen can be viewed as suitable model organisms for both pathogenic Gram-positive and Gram-negative bacteria. Ampicillin and DMSO were used as positive and negative controls, respectively. The MIC values of the plant extracts obtained using the micro-dilution methods are presented in (Table 3). Interestingly, the plant extracts tested herein showed antibacterial activity only against Gram-positive bacteria, with MIC values varying from 195 to 1562µg/mL, while Gram-negative strains were not affected at all. The higher sensitivity of the Gram-positive bacteria compared to Gram-negative bacteria could be attributed to their differences in cell envelope components. Gram-positive bacteria have an external peptidoglycan layer, which only is a permeable and thus ineffective barrier against toxic compounds Mallik [51]. Extracts of *Acacia nilotica* and *Punica granatum* (pomegranate) peals showed the highest activities against *B. aquimaris* MB 2011 with a MIC value of 195µg/mL. The antibacterial activities of these two plants against Streptococcus *viridans*, *S. aureus*, *E. coli*, *B. subtilis*, *Shigella sonnei* and *Salmonella typhimurium* were reported in previous studies [52,53] However, the reference antibiotic as a positive control showed variable inhibitory activity on the all strains of bacteria with MIC values ranging from 3.9 to 250µg/mL (Table 3). No inhibition zone was detected for the negative control (DMSO).

Table 3: MIC of the plant extracts on the studied bacterial species.

Plant Extract/ Control	Part	MIC (µg/mL)					
		B. Subtilis S168	B. Quimaris MB 2011	C. Michiganensis GSPB 390	E. coli DH 5α	E. Amylovora 1189	P. Syringae pv tomato DC 3000
H. sabdariffa	T	> 1000	> 1000	390	> 1000	125000	> 1000
A. nilotica	F	1562	195	781	1562	> 1000	781
A. bombacaceae	F	> 1000	3125	781	> 1000	> 1000	> 1000
Z. spina-christi	L	> 1000	1562	781	> 1000	> 1000	> 1000
Z. spina-christi	F	> 1000	781	6250	> 1000	> 1000	> 1000
B. aegyptiaca	B	> 1000	781	1562	> 1000	> 1000	> 1000
B. aegyptiaca	S	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
B. aegyptiaca	L	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
S. oleraceus	Wp	> 1000	> 1000	1562	> 1000	25000	> 1000
A. maritima	Wp	> 1000	781	781	> 1000	> 1000	> 1000
I. coccinea	S	1562	391	781	> 1000	> 1000	> 1000
I. coccinea	L	1562	> 1000	781	> 1000	> 1000	> 1000
O. basilicum	Ap	> 1000	1562	> 1000	> 1000	> 1000	> 1000
M. pigra	S	> 1000	781	> 1000	> 1000	> 1000	> 1000
M. pigra	L	> 1000	781	> 1000	> 1000	> 1000	1562
A.senegalensis	S	781	1562	390	> 1000	> 1000	> 1000
A. saenegalensis	L	> 1000	> 1000	1562	> 1000	> 1000	> 1000
G.tenax	F	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
C. moschata	D	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
C. roseus	L	781	> 1000	781	> 1000	> 1000	> 1000
B.madagascariensis	O	> 1000	390	3125	> 1000	> 1000	25000
C. oblonga)	F	> 1000	1562	> 1000	> 1000	> 1000	> 1000
P. granatum	P	1562	195	390	> 1000	> 1000	3125
P. granatum	J	> 1000	> 1000	390	> 1000	> 1000	> 1000
C. arabica	N	> 1000	> 1000	390	> 1000	> 1000	> 1000
T. cacao	N	> 1000	781	390	> 1000	> 1000	> 1000
C. sinensis	L	> 1000	781	390	3125	> 1000	> 1000
Ampicillin	-	< 195	< 3.9	< 3.9	15.6	250	62.5
DMSO	-	-	-	-	-	#VALUE!	-

(-): Inactive; L: Leaves; S: Stem; B: Bark; Wp: Whole plant; F: Fruits; P: Peels; D: Seeds; T: Petals; O: Pods; Ap: Aerial part; J: Juice; N: Beans.

Using agar diffusion assay, we have also investigated the antimicrobial activities of some of the crude extracts and the positive control ampicillin against the Gram-negative bacterium *E. coli* DH5α and Gram-positive bacterium *B. subtilis*

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S168. Surprisingly, some of the plant extracts showed growth inhibitory effects on both tested strains (Figure 3). Regarding the Gram-positive bacterium, *B.aegyptiaca* bark extract exhibited the highest activity (4.65±0.07mm). These findings were in agreement with previous reports [54,55]. While, for Gram-negative bacterium, the arial part extract of *O. basilicum* showed the strongest inhibitory effect (4.23±0.03mm). The positive control (ampicillin, 50mg/mL) showed zone of inhibition 18.5±0.7mm against *B. subtilis* and 16.0±0.6mm against *E. coli*. No inhibition zone was detected for the negative control (DMSO).

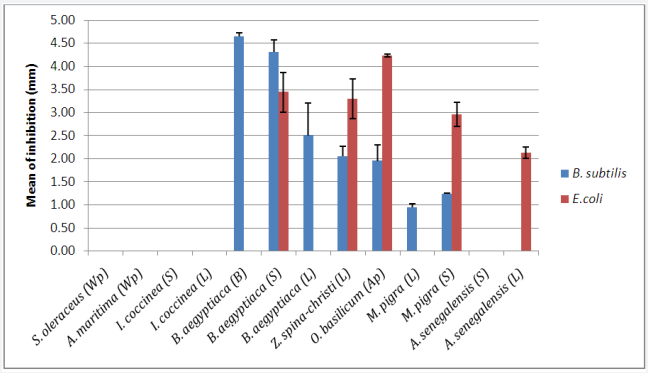

Figure 3: Inhibition zones as means ± SD generated by different plant extracts against bacterial species tested.

Phenolic compounds found in medicinal plants have been extensively studied against a wide range of microorganisms, and among them chlorogenic acids, flavanols and tannins received more interest due to their broad spectrum and the fact that most of them process antimicrobial properties [54-56]. Consequently, we have evaluated the *in vitro* antibacterial activities of some phenolic compounds against the selected bacterial strains (Table 4). Generally, the antibacterial activities the pure compounds were found to be comparatively higher than that of crude extracts. Additionally, Gram-positive bacteria were found to be more susceptible to the phenolic compounds than Gram-negative bacteria. Among the tested bioactive compounds, phloretin and resveratrol showed the strongest inhibitory activities against the all Gram-positive bacteria with MICs ranged from 9 to 125µg/mL (Table 4), followed by luteolin 7-O-glucoside and then epigallocatechin gallate (EGCG) (MICs 62 to 625µg/mL), whereas the MIC values of chlorogenic acids ranged from 260 to 540µg/mL, and therefore showed comparably low inhibitory activity. Nevertheless, the antibacterial activity of chlorogenic acids was already documented in previous studies [55,57]. The hydroxyl groups in the polyphenols are believed to be play an important role in the antimicrobial activity [58] because these groups can inactivate the microbial enzymes and interact with the cell membrane of bacteria to disrupt membrane structures and causing leakage of cellular components [59,60] (Figure 3).

Table 4: MIC of the pure phenolic on the studied bacterial species.

Compound/ control	MIC (µg/mL)					
	B. subtilis	*B. quimaris*	*C. michiganensis*	*E. coli*	*E. amylovora*	*P. syringae pv tomato*
	S168	MB 2011	GSPB 390	DH 5α	1189	DC 3000
3,4-di-O-caffeoylquinic	540	540	540	> 1000	> 1000	> 1000
4,5-di-O-caffeoylquinic	540	540	> 1000	> 1000	> 1000	> 1000
3,5-di-O-caffeoylquinic	540	540	> 1000	> 1000	> 1000	> 1000
3,4,5-tri-O-caffeoylquinic acid	540	540	> 1000	> 1000	> 1000	> 1000
1,3-di-O-caffeoylquinic acid (cynarin)	260	> 1000	> 1000	> 1000	> 1000	> 1000
5-O-caffeoylquinic acid	5000	> 1000	> 1000	> 1000	> 1000	> 1000
Phloretin	62	62	62	> 1000	> 1000	1000
Ellagic acid	> 1000	> 1000	> 1000	> 1000	> 1000	10000
Epigallocatechin gallate (EGCG)	78	78	625	> 1000	> 1000	78
Epicatechin (EC)	2500	1250	5000	10000	> 1000	> 1000
Tannic acid	> 1000	> 1000	> 1000	> 1000	> 1000	1250
Caffeic acid	10000	156	1250	> 1000	> 1000	5000
Quinic acid	10000	5000	10000	> 1000	> 1000	10000
Shikimic acid	10000	5000	10000	> 1000	> 1000	10000
Esculetin 6-O-glucoside (esculin)	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
Quercetin	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
Quercetin 3-O-(6-O-rhmanosyl-glucoside) (rutin)	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
Quercetin 3-O-arabinoside	300	> 1000	> 1000	> 1000	> 1000	> 1000
Luteolin 7-O-glucoside	62	> 1000	> 1000	> 1000	> 1000	> 1000
Resveratrol	125	15	9	250	500	> 1000
Apigenin	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000

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Ampicillin	<4	<4	<4	<4	250	31
DMSO	-	-	-	-	-	-

(-): Inactive

Evaluation of antiplasmodial and antitrypanosomal activities

Table 5 shows the antiplasmodial and antitrypanosomal activities of selected Sudanese medicinal plants against a chloroquine sensitive strain *P. falciparum* NF54 and *T. brucei rhodesiense* STI900 (African strain), respectively. For most of these plants, no specific studies of antitrypanosomal and antitrypanosomal activities exist in the literature. Most of the plant extracts exhibited dose dependent antiparasitic activities. These plants (Table 4) are traditionally used for the treatment of many ailments, parasitic and microbial infections including malaria, virus infections, digestive disorders, weakness, hepatic diseases, obesity, diabetes, skin infections, fever, diarrhea, insomnia, heart problems, colds, toothaches, hypertension, bronchial asthma, spasms, frequent urination, urinary tract infections and elimination of kidney stones [5,17,26-35]. This could explain the good observed inhibitory activities of the most of these extracts against the tested parasites. The extracts *S. oleraceus* and *B. aegyptiaca* were found to be the most promising ones. The plant extracts showed however weaker antiparasitic activity than that reported for *Chrysanthemum cinerariifolium* flower extract (86% inhibition against *P. falciparum* and 99% inhibition against *T. brucei rhodesiense* at test concentrations of 4.8µg/mL) [38]. Nevertheless, our findings were close to that reported by Karou et al. [61] which showed significant antimalarial activity for methanolic extract of *B. aegyptiaca* against *P. falciparum* (IC_{50} 24.56µg/mL). *I. coccinea*, *A. senegalensis* and *Z. spina-christi* extracts showed very low or no activities against *P. falciparum* NF54. On the other hand, *T. brucei rhodesiense* was sensitive towards the methanolic extracts of *S. oleraceus* (whole plant) and *I. coccinea* (stem) (38.4 and 25.5% inhibition activity at 10µg/mL, respectively), when relatively compared to the reported antitrypanosomal drug suramin (IC_{50} 0.03±0.02µg/mL) [62] (Table 5).

Table 5: Antitrypanosomal and antiplasmodial activity of selected crude plant extracts.

Plant Extract	Part	T. Brucei Rhodesiense Sti900		P. Falciparum Nf54	
		% Inhibition At 10µg/Ml	% Inhibition At 2µg/Ml	% Inhibition At 10µg/Ml	% Inhibition At 2µg/Ml
S. oleraceus	Wp	38.4	24.4	20.1	7.2
A. maritima	wp	9.7	13.6	25.7	11.4
I. coccinea	S	25.5	7.7	0	4.2
I. coccinea	L	18.2	9.6	8.1	6.1
B. aegyptiaca	B	25.3	10.4	25.9	3.8
B. aegyptiaca	S	26.9	8.1	0.6	0
B. aegyptiaca	L	18.3	25	2.6	0.7
Z. spina-christi	L	24.7	15.3	6.6	0
O. basilicum	Ap	29.1	21.8	7.5	12.4
M. pigra	L	12.3	8.4	14.8	10.6
M. pigra	S	5.3	1.9	5.2	3.6
A. senegalensis	S	9	0.2	3.7	3.3
A. senegalensis	L	7.5	3.4	0	3

Evaluation of antioxidant activities

It is believed that the antioxidant activity of plant extracts rich phenolic phytoconstituents is due to their ability to be donors of hydrogen atoms or electrons and to capture the free radicals. Scavenging activity for free radicals of 1.1-diphenyl-2-picrylhydrazyl (DPPH) has been commonly used to assess the antioxidant activity of medicinal plants and natural products. Plant extracts from Sudan were prepared for investigation of their antioxidant activities. They showed significant free radical scavenging activity at the high concentration (100µg/mL) on DPPH (Figure 4). The extracts of *M. pigra* stem, *M. pigra* leaves, O. *basilicam arial* part and *I. coccinea* leaves were the most effective radical scavengers with the inhibition of 33.4 ± 3.3%, 29.1±2.4%, 26.3 ± 1.2% and 26.7 ± 1.4%, respectively, as compared to 83.5±1.5% for gallic acid standard. Nevertheless, these crude plant extracts showed better DPPH scavenging activity than that reported for *Crataegus monogyna* fruits extract (15±1% scavenging activity at a test concentration of 100µg/mL) a flavonoid drug included in most European pharmacopeia [20]. At lower concentration (10 µg/mL) the extracts were not effective as the positive standard (Figure 4), indicating that the activity was concentration dependent. Many studies have demonstrated that the antioxidant activity is significantly affected by the phenolic constituents of the sample [20,21]. Thus, the radical-scavenging activity of the plant extracts may be attributed to their phenolic and flavonoid contents. Furthermore, the antioxidant property of *M. pigra* was in agreement with those mentioned in the literature [61,63-70] (Figure 4). Percentage inhibition as means±SD of free radical scavenging by the plant extracts.

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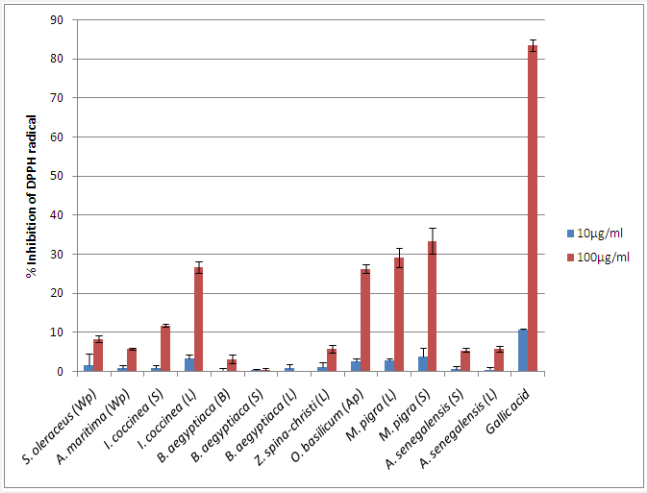

Figure 4: Percentage inhibition as means ± SD of free radical scavenging by the plant extracts.

Conclusion

In conclusion, our findings strongly support the traditional use of the studied Sudanese plants in the treatment of bacterial and parasitic infections. The results revealed that some of these plants, such as *A. nilotica*, *O. basilicam*, *Z. spina-christi*, *B. aegyptiaca*, *S. oleraceus*, *P. granatum*, *M. pigra* and *I. coccinea* have the potential to be investigated further to identify the antioxidative, antiplasmodial, antitrypanosomal, and antibacterial metabolites in these plants. The study also reports on the antibacterial activities of some naturally occurring phenolic compounds. Among the tested phytochemicals, phloretin, resveratrol, luteolin 7-O-glucoside and epigallocatechin gallate showed the highest antimicrobial activities. By means of preparative HPLC, HPLC-ESI-TOF, HPLC-ESI-MS^n, ^{1}H-NMR and ^{13}C-NMR, thirteen phytoconstituents were isolated and identified in the methanolic extracts of *Z. spina-christi*, *S. oleraceus* and *H. sabdariffa* including chlorogenic acids, flavonoid glycosides, coumarins and derivatives. The results of this study highlight the importance of the Sudanese medicinal plants as potential source of plant derived antimicrobial and antiparasitic drugs. However, pharmacological and toxicological studies will be necessary to confirm this hypothesis.

Conflict of interest

The authors disclose that there is no conflict of interest.

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