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Phenolic composition and prospective anti-infectious properties of *Atriplex lindleyi*

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ABSTRACT

Objective: To investigate the antiplasmodial, antimicrobial, radical scavenging effects and to identify the phenolic constituents of *Atriplex lindleyi* (*A. lindleyi*).

Methods: *A. lindleyi* extracts and some isolated compounds were tested *in vitro* against the chloroquine-resistant strains of *Plasmodium falciparum*. The radical scavenging activity was quantified by using 2,2-diphenyl-2-picrylhydrazyl nitrogen-centered free radical. The IC₅₀ of each extract was compared with references. The *in vitro* anti-infectious activity of extracts was evaluated against representative Gram-positive and Gram-negative bacterial strains [*Staphylococcus aureus* CIP 4.83, *Enterococcus hirae* CIP 5855, *Pseudomonas aeruginosa* (*P. aeruginosa*) CIP 82118, *Escherichia coli* CIP 53126], and fungal species [(*Candida albicans* (*C. albicans*) IP 48.72, *Aspergillus niger* IP 1431.83)]. Ethanol extract was investigated for chemical composition through column and high performance liquid chromatography. The isolated compounds were identified by mass spectrometry and nuclear magnetic resonance.

Results: Quercetin-7-*O*-arabinopyranoside-3-*O*-neohesperidosides (1), quercetin-3-*O*-arabinopyranosyl(1→6)glucopyranoside (2), quercetin-3-*O*-glucopyranoside-7-*O*-rhamnopyranoside (3), quercetin-3-*O*-glucopyranoside-7-*O*-arabinoside (4), schaftoside (5), quercetin-7-*O*-glucopyranoside (6) were isolated for the first time from the ethanol extract of *A. lindleyi* aerial parts in addition to isorhamnetin-3-*O*-β-glucopyranoside (7) and quercetin (8). The extracts exhibited moderate antiplasmodial activity with IC₅₀ ranging from 10–50 μg/mL. Quercetin was the most potent compound with IC₅₀ of 9 μg/mL. *P. aeruginosa* and *C. albicans* were the most susceptible organisms.

Conclusions: The study implies that *A. lindleyi* can contribute to the fight against malaria, and be useful as prophylactic against *C. albicans* and *P. aeruginosa*.

Keywords:

Atriplex

Antibacterial

Antifungal

Antiplasmodial

Chenopodiaceae

Flavonoids

1. Introduction

Malaria is one of the most dangerous tropical parasitic diseases. About 198 million cases of malaria infection and an estimated 584 million deaths were reported in 2013, mostly among children living in Africa where almost one child dies every minute[1]. It also poses a risk to travellers and immigrants, with imported cases increasing in non-endemic areas[2]. Therefore, endemic countries started to adopt artemisinin-based combination therapies for uncomplicated malaria[3]. However, in the Greater Mekong Subregion, *Plasmodium*

falciparum (*P. falciparum*) has become resistant to almost all available antimalarial medicines. In addition, there is a real risk that multidrug resistance will emerge soon in other parts of the subregion as well[4]. Therefore, the current research aimed at the development of new antimalarial agents.

The genus *Atriplex* comprises of 200 species and belongs to the subfamily Chenopodiaceae. Although this genus is considered as common weeds, it has always been used as folk remedy. In the Arab world, *Atriplex halimus* is used as a laxative, stomach pain killer, against intestinal worms, to regulate gall bladder excretions and to treat chest ailments. The Bedouin tribes in the Negev use the plant for treating muscular pain and intestinal diseases in animals, to feed sheep and goats, and to flavor cooking. The leaves are edible but too salty to be eaten alone[5]. The chemical composition of *Atriplex* species is very complicated as it contains flavonol as the major components[6,7], triterpene saponins[8,9], alkaloids and amino acids[10], coumarins and ecdysterols[11]. Therefore, *Atriplex*

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species have been reported as cytotoxic[9,12], antimicrobial[13], antioxidant[14], antidiabetic[15], and to reduce male fertility in rats[11].

Of the *Atriplex* species, *Atriplex lindleyi* Moq. (*A. lindleyi*) synonym: *Atriplex halimoides*, known as Lindley's saltbush, is widely spread in Egypt, especially in dry areas. The hepatorenal protective potential of Lindley's saltbush was evaluated[16], and its antidiabetic, antihepatotoxic, antimicrobial and anti-inflammatory activities were reported[17,18]. In addition, several compounds were isolated such as flavonoids, quercetin, quercetrin, isoquercetin and isorhamnetin-3-*O*-glucopyranoside besides lupeol, eccesterol, 3,23-dihydroxybetulin, 20-hydroxyecdysone, olean-12-en-3,11-dione, β -amyrenone, erythrodiol and cholesterol[16-18]. The present work deals with the identification of phenolic constituents and the investigation of the antiplasmodial, antimicrobial and radical scavenging effect of *A. lindleyi* extracts.

2. Materials and methods

2.1. General experiment

Ultraviolet spectra were recorded by using UV-vis double beam UWD-3500 spectrophotometer (Labomed, Inc. USA). Electrospray ionization mass (ESIMS) was obtained from an LCQ Advantage Thermo/Finnigan spectrometer (Thermo Finnigan, USA). Nuclear magnetic resonance (NMR) spectra were measured on JEOL EX-500 spectrometer (Tokyo, Japan) with 500 MHz for $^1\text{H-NMR}$ and 125 MHz for $^{13}\text{C-NMR}$ by using methyl sulfoxide- d_6 as solvent and as internal reference. Analytical thin layer chromatography (TLC) was done on TLC cellulose and silica gel 60 F₂₅₄ (200 mm layer thickness) (Merck; Darmstadt, Germany). Chromatograms were visualized under UV light at 366 nm before and after sprays of aluminium chloride reagent.

The phenolic acids were analyzed by using high performance liquid chromatography (HPLC), HPLC Agilent 1100 series (Waldborn, Germany), equipped with quaternary pump (G1311A), degasser (G1322A); the column used was Zorbax 300SB C18 (Agilent Technologies, USA). Injection was carried out at wave length of 280 nm for separation of phenolic compounds. Reference compounds were purchased from Sigma-Aldrich (Steinheim, Germany), and column chromatography for fractionation was employed by using Polyamide 6S (Riedel-de-Haen AG, Seelze Haen AG, D-30926 Seelze Hanver, Germany). The purification was performed on Sephadex LH-20 (Pharmacia, Sweden) column. All chemicals of analytical grade were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Solvents were purchased from Merck (Darmstadt, Germany).

2.2. Plant material

A. lindleyi Moq. aerial parts were collected in March from Road of Suez, Egypt. A voucher specimen (No. 954) was kept in the Herbarium of National Research Centre.

2.3. Extraction and isolation

Dried powdered aerial parts of *A. lindleyi* (350 g) were successively extracted with petroleum ether (40–60 °C), chloroform and 70% aqueous ethanol (23.0, 12.5 and 12.0 g), respectively. The aqueous ethanol extract was subjected to reversed phase liquid chromatography on polyamide eluted with water gradient and decreased polarity with methanol. Fractions of 100 mL were collected and monitored on TLC silica plates by using ethyl acetate: formic acid: acetic acid: water (100:11:11:27 v/v/v/v) as developing solvent. To obtain three substantial fractions, major compounds

were isolated and purified on Sephadex LH20 subcolumn. Purity of isolated compounds was inspected through two-dimensional TLC cellulose plates eluted with solvent systems: (1) 15% HOAc (water: acetic acid 85:15 v/v), (2) BAW (butanol: acetic acid: water 4:1:5 v/v, upper layer).

2.4. Acid hydrolysis

Acid hydrolysis was performed by using 6% aqueous hydrochloric acid at 100 °C for 2 h. For aglycone detection, the final mixture was extracted with ethyl acetate, then the aqueous layer was neutralized for determination of the released sugar moiety by using silica gel plates with the mixture of *n*-propanol: ethyl acetate: water (7:2:1 v/v/v); aniline phthalate was employed as a spray for color detection of the sugars[19].

2.5. Characterizations of compounds

Characterizations of compound 1 were UV (λ_{max}) nm: (MeOH): 257, 269sh, 299sh, 378; (+NaOMe): 291, 367, 456; (+AlCl₃): 277, 302sh, 436; (+AlCl₃/HCl): 274, 297, 360sh, 403; (+NaOAc): 286, 375, 426; (+NaOAc/H₃BO₃): 261, 384; ESIMS (-ve) *m/z*: 741 [M-H]⁻, 609 [M-H-132]⁻, 489 [M-H-132-120]⁻, 301 [M-H-132-308]⁻.

Characterizations of compound 2 included UV (λ_{max}) nm: (MeOH): 257, 269sh, 299sh, 358; (+NaOMe): 272, 327, 410; (+AlCl₃): 274, 303sh, 433; (+AlCl₃/HCl): 271, 299, 364sh, 403; (+NaOAc): 271, 325, 393; (+NaOAc/H₃BO₃): 262, 299, 387; $^1\text{H-NMR}$ δ : 12.64 (1H, s, 5-OH), 7.54 (1H, d, *J* = 2.5 Hz, H-2'), 7.53 (1H, dd, *J* = 8.5, 2.5 Hz, H-6'), 6.83 (1H, d, *J* = 8.5 Hz, H-5'), 6.36 (1H, d, *J* = 2.5 Hz, H-8), 6.15 (1H, d, *J* = 2.5 Hz, H-6'), 5.33 (1H, d, *J* = 6.8 Hz, H-1''), 5.2 (1H, d, *J* = 7.6 Hz, H-1'''); $^{13}\text{C-NMR}$ δ : 157.9 (C-2), 133.7 (C-3), 177.8 (C-4), 161.7 (C-5), 99.2 (C-6), 164.9 (C-7), 94.1 (C-8), 157.2 (C-9), 104.3 (C-10), 122.1 (C-1'), 115.8 (C-2'), 145.3 (C-3'), 149.1 (C-4'), 115.6 (C-5'), 121.5 (C-6'), 103.2 (C-1''), 74.4 (C-2''), 76.8 (C-3''), 70.5 (C-4''), 77.3 (C-5''), 67.8 (C-6''), 101 (C-1'''), 70.9 (C-2'''), 72.9 (C-3'''), 70.9 (C-4'''), 65.4 (C-5'''). ESIMS (-ve) *m/z*: 595 [M-H]⁻.

Characterizations of compound 3 were as follows: ESIMS (-ve) *m/z*: 609 [M-H]⁻, 447 [M-H-glucose]⁻, 463 [M-H-rhamnose]⁻, 301 [M-H-glucose-rhamnose]⁻; $^1\text{H-NMR}$ δ : 12.64 (1H, s, 5-OH), 7.62 (1H, d, *J* = 2.5 Hz, H-2'), 7.60 (1H, dd, *J* = 8.5, 2.5 Hz, H-6'), 6.85 (1H, d, *J* = 8.5 Hz, H-5'), 6.80 (1H, d, *J* = 2.5 Hz, H-8), 6.43 (1H, d, *J* = 2.5 Hz, H-6), 5.49 (1H, d, *J* = 6.9 Hz, H-1'), 5.55 (1H, s, H-1''), 1.12 (3H, d, *J* = 6.0 Hz, -CH₃); $^{13}\text{C-NMR}$ δ : 156.8 (C-2), 133.7 (C-3), 177.7 (C-4), 160.9 (C-5), 99.5 (C-6), 161.7 (C-7), 94.4 (C-8), 156.8 (C-9), 105.7 (C-10), 121.8 (C-1'), 115.3 (C-2'), 145.0 (C-3'), 148.9 (C-4'), 116.4 (C-5'), 121.1 (C-6'), 100.8 (C-1''), 74.2 (C-2''), 76.6 (C-3''), 70.3 (C-4''), 77.8 (C-5''), 61.1 (C-6''), 99.5 (C-1'''), 70.0 (C-2'''), 70.2 (C-3'''), 71.7 (C-4'''), 69.9 (C-5'''), 18.0 (C-6''').

Characterizations of compound 4 were UV (λ_{max}) nm: (NaOMe): 269, 400; (AlCl₃): 275, 330sh, 433; (AlCl₃ + HCl): 269, 300sh, 360sh, 400; (NaOAc): 262, 408 and (NaOAc + H₃BO₃): 260, 378; ESIMS (-ve) *m/z*: 595 [M-H]⁻, 463 [M-H-arabinose]⁻, 301 [M-H-glucose-arabinose]⁻.

Characterizations of compound 5 included UV (λ_{max}) nm: (MeOH): 271, 338; $^1\text{H-NMR}$ δ : 8.02 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.91 (2H, d, *J* = 8.65 Hz, H-3', 5'), 6.81 (1H, s, H-3); 6-C- β -Glc: 4.73 (1H, d, *J* = 9.8 Hz, H-1''); 8-C- α -Ara: 4.83 (1H, d, *J* = 9.7 Hz, H-1'''); $^{13}\text{C-NMR}$ δ : 182.3 (C-4), 163.6 (C-2), 161.2 (C-7), 161.2 (C-5), 160.8 (C-4'), 153.4 (C-9), 128.8 (C-2', 6'), 121.3 (C-1'), 116.0 (3', 5'), 109.3 (C-6), 103.7 (C-8), 103.0 (C-10), 102.5 (C-3); 6-C- β -Glc: 74.3 (C-1), 68.4 (C-2), 79.4 (C-3), 70.3 (C-4), 79.0 (C-5), 60.8 (C-6); 8-C- α -Ara: 74.9 (C-1), 69.8 (C-2), 73.7 (C-3), 70.0 (C-4), 70.3 (C-5). ESIMS (*m/z*): 563 [M-H]⁻, 473 [M-90]⁻, 443 [M-120]⁻, 383 [M-180]⁻, 353 and 297.

Compound 6 was observed in yellow fluorescence under UV at λ 360 nm. Its characterizations were as follows: UV (λ_{\max}) nm: (MeOH): 259, 267sh, 378; (+NaOMe): 291, 241sh, 367, 456; (+AlCl₃): 275, 259sh, 332sh, 439; (+AlCl₃/HCl): 271, 300sh, 363, 426; (+NaOAc): 286, 375, 426; (+NaOAc/H₃BO₃): 261, 289, 384; ESIMS (-ve) *m/z*: 463 [M-H]⁻, *m/z* 301 [M-H-162]⁻; ¹H-NMR δ : 12.60 (1H, s, 5-OH), 7.29 (1H, d, *J* = 1.5 Hz, H-2'), 7.24 (1H, d, *J* = 8.4 Hz, H-6'), 6.85 (1H, d, *J* = 8.4 Hz, H-5'), 6.36 (1H, br s, H-8), 6.19 (1H, brs, H-6), 5.10 (1H, d, *J* = 7.7 Hz, H-1''); ¹³C-NMR δ : 147.9 (C-2), 134.6 (C-3), 181.2 (C-4), 161.8 (C-5), 99.3 (C-6), 160.1 (C-7), 94.1 (C-8), 157.7 (C-9), 104.4 (C-10), 121.6 (C-1'), 115.9 (C-2'), 145.7 (C-3'), 149.0 (C-4'), 116.1 (C-5'), 121.2 (C-6'), 100.2 (C-1''), 74.5 (C-2''), 77.6 (C-3''), 71.3 (C-4''), 75.4 (C-5''), 65.8 (C-6'').

Characterizations of compound 7 were UV (λ_{\max}) nm: (MeOH): 252, 266sh, 352; (+NaOMe): 270, 330, 416; (+AlCl₃): 262, 298sh, 400; (+AlCl₃/HCl): 262, 299, 366sh, 400; (+NaOAc): 274, 325, 394; (+NaOAc/H₃BO₃): 254, 268, 356; ESIMS (-ve) *m/z*: 477 [M-H]⁻, 300 [M-H-glucose-CH₃]⁻; ¹H-NMR δ : 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.89 (1H, d, *J* = 8.0 Hz, H-5'), 7.57 (1H, dd, *J* = 2.0 and 7.5 Hz, H-6'), 8.02 (1H, d, *J* = 2.0 Hz, H-20), 3.95 (3H, s, OMe), 5.32 (1H, d, *J* = 7.6 Hz, H-1'').

Characterizations of compound 8 included ESIMS (-ve) *m/z* 301 [M-H]⁻; ¹H-NMR δ : 6.17 (1H, d, *J* = 2.0 Hz, H-6), 6.37 (1H, d, *J* = 2.0 Hz, H-8), 6.87 (1H, d, *J* = 8.0 Hz, H-5'), 7.62 (1H, dd, *J* = 2.0, 7.5 Hz, H-6), 7.73 (1H, d, *J* = 2.0 Hz, H-2').

2.6. HPLC of phenolic acids

To prepare the fraction containing phenolic compounds, one gram of the aqueous ethanol extract was re-extracted with normal butanol in separating funnel, and the butanol fraction was evaporated. The residue was re-dissolved in methanol, then shaken with ether, allowed to stand. Finally, the supernatant was filtered, dried and subjected to HPLC as described above, by using mobile phase and acetonitrile: phosphate buffer (86:14 v/v) at flow rate of 1 mL/min.

2.7. Antiplasmodial assay

Antiplasmodial activity was tested according to the protocol previously reported[20]. Roswell Park Memorial Institute 1640 medium [BioWhittaker, Cambrex (catalogue No. BE12-702F), Belgium] containing L-glutamine (BioWhittaker, catalogue No. BE17-605E), 25 mmol/L 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethanesulfonic acid (BioWhittaker, catalogue No. 17-737F), and 10% human serum (Etablissement Français du Sang, Toulouse, France), was used to cultivate *P. falciparum*. Human red blood cells (RBCs) (group O \pm) were obtained from Etablissement Français du Sang. They were extensively washed with Roswell Park Memorial Institute medium to remove plasma and leucocytes. Leucocyte-free erythrocytes were stored at 50% hematocrit for a maximum period of 21 days. *P. falciparum* asexual blood-stage parasites were propagated by incubation at 37 °C in *P. falciparum* culture media at 3%–5% hematocrit in a controlled atmosphere (5% CO₂, 100% relative humidity).

Parasitized RBCs were maintained in 25 cm² culture flasks (TPP, Switzerland, reference No. 90025). Reference drugs, chloroquine and artesunate were obtained from Sigma (reference No. C6628) and Cambrex, respectively. Chloroquine was dissolved in culture medium and artesunate in ethanol (stock solutions of 10 mg/mL) and stored at -20 °C before tested. For extract assays, serial extract dilutions were made in *P. falciparum* culture media and added to 96-well (TPP) culture plates. Plasmodium-infected RBCs were distributed at 2% parasitaemia (2% hematocrit) in 96-well microtiter plates with different drug concentrations and incubated for 48 h at 37 °C and 5% CO₂.

[3H]-Hypoxanthine (Perkin-Elmer) was added 24 h after the beginning of the incubation. At the end of incubation (48 h), microtiter plates were frozen and thawed, and each well was harvested onto a glass-fiber filter paper. The quantity of incorporated [3H]-hypoxanthine was determined with a β -counter (1450-Microbeta Trilux, Wallac-PerkinElmer). Growth-inhibition percentages were plotted as a semi-logarithmic function of drug concentration. The IC₅₀ values were determined by linear regression analysis on the linear segments of the curves. Assays were repeated three times for each concentration. Controls were carried out to assess the background (negative control) and parasite growth (positive control).

2.8. Antimicrobial assays

Antimicrobial activity was tested according to the method previously reported[20]. The extracts were dissolved in water/dimethylsulfoxide (DMSO) to obtain an initial concentration of 1000 μ g/mL. The resulting solutions were then diluted in microtiter plates in trypticase soja medium (Biomérieux, Craaponne, France) for bacteria and Sabouraud (Biomérieux, Craaponne, France) for fungi and yeast. Gram-positive strains, *Staphylococcus aureus* (*S. aureus*) CIP 4.83 and *Enterococcus hirae* (*E. hirae*) CIP 5855, and Gram-negative strains, *Pseudomonas aeruginosa* (*P. aeruginosa*) CIP 82118 and *Escherichia coli* CIP 53126, were used for the antimicrobial assays. For the antifungal assay, cultures of the yeast *C. albicans* IP 48.72 and mould *Aspergillus niger* (*A. niger*) IP 1431.83 were employed. Strains were obtained from the collection of the Pasteur Institute (Paris, France). Microbial suspensions were prepared in sterile distilled water to obtain final inoculums of 10⁶ cells/mL and 10⁵ spores/mL for bacteria and fungi, respectively.

Minimal inhibitory concentrations (MICs) and minimal germicidal concentrations [(minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC)] were determined after incubation of the bacterial strains at 37 °C and the fungal strains at 22.5 or 30.0 °C for 24 h (36 h for *A. niger*) in the presence of serial dilutions of the test compounds. The MIC was defined as the concentration of compound at which no macroscopic sign of cellular growth was detected in comparison to the control without antimicrobial compound. The MBC/MFC was determined by sub-cultivation on corresponding agar plates after incubation of the bacterial strains at 37 °C for 24 h and fungal strains at 22.5 or 30.0 °C for 48 h.

MBC/MFC was defined as the concentration of compound at which no macroscopic sign of cellular growth was detected in comparison to the control without antimicrobial compound on subculturing. All the experiments were carried out in duplicate at each concentration. In order to ensure that DMSO *per se* did not interfere with the antimicrobial activity evaluation of the products under assay, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments.

2.9. 2,2-diphenyl-1-picrylhydrazyl assay

The antioxidant activity was evaluated by using the method previously reported[20]. The stable 2,2-diphenyl-2-picrylhydrazyl nitrogen-centered free radical (DPPH[•]). DPPH in ethanol (300 μ mol/L, 100 μ L) was added to 200 μ L of the test compounds at different concentrations in the appropriate solvents (MeOH, EtOH, MeOH/H₂O 2:1, v/v). Each mixture was then mixed thoroughly and the absorbance was recorded every 5 min for 30 min, by using a microplate reader (UV Max, Molecular Devices). The decrease in DPPH[•] absorbance (at λ 530 nm) was monitored. DPPH[•] solution (100 μ L) in the respective solvent (200 μ L) served as the blank.

All tests were performed in triplicate. The radical scavenging

activity of the samples (antioxidant activity) was expressed in terms of IC₅₀ (concentration in mg/L required for a 50% decrease in DPPH[•] absorbance). A plot of absorbance vs. concentration was made to establish the standard curve and to calculate the IC₅₀. Ascorbic acid and Trolox (water-soluble form of vitamin E) were used as positive controls.

3. Results

3.1. Column chromatography

Column chromatography of aqueous ethanol extract afforded eight flavonoids, namely, quercetin-7-*O*-arabinoside-3-*O*-neohesperidosides (1), quercetin-3-*O*-arabinopyranosyl(1→6)-glucopyranoside (2), quercetin-3-*O*-glucopyranoside-7-*O*-rhamnopyranoside (3), quercetin-3-*O*-glucoside,7-*O*-arabinose (4), schaftoside (5), quercimeritrin (6), isorhamnetin-3-*O*-glucoside (7) and quercetin (8). Their structural elucidation was established by comparing the UV, ESIMS and/or NMR spectral data with reference data from available literature[21-23]. While the absolute configuration of the sugar was elucidated through chemical degradation and comparison with an authentic standard. To the best of our knowledge, compounds 1-6 were isolated from *A. lindleyi* aerial parts for the first time.

In the case of compound 1, the negative mode ESI of compound 1 showed molecular ion at *m/z* 741 [M-H]⁻. The MS data showed fragment ion at *m/z* 609 [M-H-132]⁻, 489 [M-H-132-120]⁻, and 301 [M-H-132-308]⁻, indicating successive elimination of pentose, rhamnose and hexose, respectively. In addition, fragment ion at *m/z* 308 was indicative of compound having the disaccharide structure, rutinose or neohesperidose linked through an -*O*-glycosidic bond. The fragmentation patterns of flavonoid *O*-neohesperidosides were more complicated in comparison with their rutinoside analogues. A major difference was found in the [M-H-120]⁻ ion in the MS2 spectrum, which was a common feature of all the flavonoid *O*-neohesperidosides[24]. The low intensity of fragment ion *m/z* 609 due to loss of pentose indicates its position at C7[21,25]. The chromatographic behavior and UV supported a 3,7-glycosylated flavonolstructure. Additional confirmation was obtained by acid hydrolysis and co-chromatography with authentic sugars. These emphasized that this compound was identified as quercetin-7-*O*-arabinoside-3-*O*-neohesperidosides.

Compound 2 showed purple spot when examined under UV at λ 365 nm, suggesting substituted 3 hydroxyl group, which was confirmed by UV-shift reagents. The ¹H-NMRδ showed the expected signal in the aromatic region for -3-*O*- substituted quercetin, signals of two anomeric protons of sugar doublet at δH 5.2 (*J* = 7.65 Hz) and 5.3 (*J* = 6.85 Hz); their coupling constants matched the values of β-D-glucopyranoside and α-L-arabinopyranoside, respectively[21-23]. This was confirmed by the observed two anomeric carbon of sugar at 101.3 and 103.2 ppm in ¹³C-NMRδ and the appearance of molecular ions in ESI/MS at *m/z* 595 [M-H]⁻ and fragment ion at *m/z* 301 [M-H-294]⁻ due to loss of hexose and pentose units. Acid hydrolysis indicated the presence of glucose and arabinose. The ¹³C-NMR spectra showed significant down field shift of 6 ppm for the C6 signal of glucose due to interglycosidic linkage indicating that the glucopyranoside unit directly attached to the aglycone and arabinopyranoside was a terminal sugar. Altogether, compound 2 was identified as quercetin 3-*O*-α-L-arabinopyranosyl(1"→6")-β-D-glucopyranoside[26].

For compound 3, the MS, ¹H-NMR and ¹³C-NMR data were in agreement with those reported for quercetin-3-*O*-glucopyranoside-7-*O*-rhamnopyranoside[27].

In the situation of compound 4, the UV absorbance of compound with shift reagents indicated a flavonol moiety substituted at C7 and C3. ESI/MS of compound 4 showed a mass molecular ion at *m/z*

m/z 595 [M-H]⁻. The MS2 fragment ion at *m/z* 463 [M-H-132]⁻ was corresponding to the loss pentose moiety. Further, MS3 fragment ion was observed at *m/z* 301 [M-H-132-162]⁻ indicating the loss of a hexose unit. The sugars were identified by acid hydrolysis and co-chromatography with authentic sugars, arabinose and glucose. The intensity of the fragment resulting from loss of hexose was higher than that obtained from loss of pentose, indicating glucose attached to C3 meanwhile arabinose was at C7. The compound was identified as quercetin-3-*O*- glucoside,7-*O*-arabinoside[25].

For compound 5, all data were in accordance with those of apigenin-6-*C*-glucopyranoside-8-*C*-arabinopyranoside (schaftoside)[28].

For compound 6, chemical shifts values at δ^H6.44 for H-6, δ^H 6.81 for H-8, δ^C 147.9 (C-2) and δ^C 160.1 for (C-7) suggested 7-*O*-glycosylated flavonoids, while anomeric proton at δ 5.10 with *J* = 7.7 Hz indicated the β configuration. Results of UV, MS, ¹H-NMR and ¹³C-NMR were in agreement with those previously reported in the literature of quercetin-7-*O*-glucopyranoside (quercimeritrin)[21-23].

For compound 7, the UV, ESIMS, ¹H-NMR results were corresponding to those of isorhamnetin-3-*O*-glucoside[21-23].

For compound 8, ESIMS and ¹H-NMR results matched those reported to quercetin[21-23].

3.2. HPLC of phenolic acid fraction

HPLC analysis of phenolic acid fraction led to identification of five compounds which were compiled in Table 1. Ellagic acid (26.4%) and catechin (31.3%) were predominant ones.

Table 1

HPLC analysis of phenolic acids.

Compounds	Retention time (min)	Relative (%)
Gallic acid	3.00	5.3
Caffeic acid	3.50	15.4
Ellagic acid	4.00	26.4
Catechin	6.90	31.3
Coumaric acid	11.17	21.1

3.3. Antiplasmodial and cytotoxic activities

The antiplasmodial effect of tested extracts and compounds against *P. falciparum* was expressed as concentration that killed 50% of parasites (IC₅₀) in Table 2. All extracts possessed moderate lethal effect IC₅₀ ranging from 10 to 50 μg/mL. Among the tested flavonoids, compound 8 (IC₅₀ = 9 μg/mL) was the most potent followed by compound 6 then compound 2 and finally compound 3. Extracts exhibited cytotoxicity with 50% cytotoxicity concentration (CC₅₀) values ranging from 27–33 μg/mL on the mammalian MCF-7 cell line.

Table 2

Antiplasmodial activity and cytotoxicity of *A. lindleyi* extracts and some isolated compounds.

Sample	IC ₅₀ ^a ± SD (μg/mL)	CC ₅₀ ± SD (μg/mL) ^b	Selectivity index ^c
	FcB1 strain	MCF-7	MCF-7/FcB1
Pet ether extract	33	27.00	0.82
Chloroform extract	28.5000 ± 0.5000	32.00	1.02
Ethanol extract	33.0000 ± 1.0000	30.00	0.91
Compound 2	63	Not tested	-
Compound 3	> 100	Not tested	-
Compound 6	33	Not tested	-
Compound 8	9	Not tested	-
Chloroquine (n = 5)	0.0720 ± 0.0150	9.70	134.00
Sodium artesunate	0.0024 ± 0.0012 ^e	3.97 ^d	1654.00

Values were expressed as mean percentage of three replication ± SD; ^a: Drug concentration that killed 50% of FcB1 strain; ^b: Drug concentration (CC₅₀) needed to cause 50% decrease of the cellular viability of MCF-7; ^c: Cytotoxicity/antiplasmodial activity ratio; ^d: Nepveu *et al.*, 2010[29].

Table 3Antibacterial and antifungal activity of *A. lindleyi* extracts. µg/mL.

Sample	<i>S. aureus</i>		<i>P. aeruginosa</i>		<i>E. hirae</i>		<i>Escherichia coli</i>		<i>A. niger</i>		<i>C. albicans</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
Pet ether extract	125	500	62.5	250	125.00	> 500.00	125.00	500.00	125.00	-	62.50	125.00
Chloroform extract	> 500	> 500	62.5	125	125.00	> 500.00	125.00	500.00	125.00	-	62.50	125.00
Ethanol extract	125	500	125.0	250	125.00	> 500.00	125.00	500.00	125.00	-	62.50	125.00
Nalidixic acid	125	125	> 125.0	> 125	6.25	6.25	3.12	3.12	-	-	-	-
Bifonazole	> 125	> 125	> 125.0	> 125	> 125.00	> 125.00	> 125.00	> 125.00	3.12	-	125.00	> 125.00
Clotrimazole	> 125	> 125	> 125.0	> 125	> 125.00	> 125.00	> 125.00	> 125.00	1.56	-	3.12	6.25

3.4. Antimicrobial activity

The antimicrobial activities were summarized in Table 3. The results showed that extracts demonstrated antibacterial activity with MIC and MBC ranging from 62–125 µg/mL and 125–500 µg/mL, respectively, exhibiting similar inhibitory effect on *E. hirae* and other three germs. Petroleum ether and chloroform extracts were more active on *P. aeruginosa* than reference drugs (nalidixic acid, bifonazole, clotrimazole).

Meanwhile, petroleum ether and aqueous ethanol extracts exhibited antimicrobial activity against *S. aureus* similar to reference drugs. On the other hand, all the extracts showed the same antifungal effect on the tested fungi. The yeast *C. albicans* was more susceptible to the extracts with MIC values of 62.5 µg/mL.

3.5. Radical scavenging ability

The IC₅₀ of extracts in DPPH absorbance represented in Table 4 revealed that the petroleum ether extract had no radical scavenging ability at the tested concentration.

Table 4The DPPH radical scavenging activity (IC₅₀) of the extracts compared with vitamin C and Trolox as standard antioxidants.

Sample	IC ₅₀ ^a (mg/L) ± SD
Pet ether extract	> 667
Chloroform extract	332.46 ± 16.21
Ethanol extract	345.70 ± 2.77
Trolox	8.36 ± 0.05
Ascorbic acid	3.80 ± 0.08

Each value was expressed as mean percentage of three replication ± SD; ^a: Concentration that inhibited DPPH absorbance by 50%.

4. Discussion

In spite of extensive studies, the structure-activity relationships for the antiplasmodial action remain unclear. Here we found that the hydrophilic extract which comprises predominantly flavonoids and phenolic acids showed radical scavenging ability and antiplasmodial activity. Our detailed investigation of the isolated compounds used as antiplasmodial showed that the aglycone with free hydroxyl groups was the most active compound and its activity decreased upon the substitution of hydroxyl groups. We also observed that the 7-*O*-substituted flavonol was relatively more active than 3-*O*-substituted and that the disubstitution at 7-*O* and 3-*O* was unfavorable to the antiplasmodial activity. From our data, it can be concluded that the free OH considerably increased the antiplasmodial activity, which is reduced when the free OH is occupied by sugar moiety. These results are supported by an earlier study^[30], which focused on the efficacy of different flavonoids and gallic acid esters of catechins

toward three important enzymes, *i.e.* β-ketoacyl-ACP-reductase, β-hydroxyacyl-ACP-dehydratase and enoyl-ACP-reductase involved in the fatty acid biosynthesis of *P. falciparum* strain. The authors found that the activity of flavonoids was attributed to the combination of two (catechol) or three hydroxyl group in ring B (regardless of an additional hydroxyl group at C-3). They also reported that the presence of the double bond between C-2 and C-3 was essential to keep the planarity structure of flavonoids with respect to activity. Another study found that a bulky substituent such as sugar moiety at C-3 of flavonol forced the ring B out of the plane of the AC-ring^[31]. These studies explained why compound 6 was relatively active compared to compound 2 while compound 3 was inactive. These results are in agreement with those deduced by Moalin *et al.* regarding the antioxidant capacity of quercetin derivatives^[31]. Another parameter is the oxidative stress which is considered beneficial for killing parasites and for weakening the adherence between infected RBCs and endothelial cells or monocytes. It was reported that compounds with high oxidation potential may be used as relatively efficient antiplasmodial agents with low mammalian cell cytotoxicity^[32]. In our study, both chloroform and ethanol extracts displayed higher radical scavenging ability, antiplasmodial activity and lower cytotoxicity than petroleum ether extract. In contrast, the petroleum ether extract showed no radical scavenging ability, relatively lower antiplasmodial activity and higher cytotoxicity than the ethanol extract. The constituents of petroleum ether extract have been recently studied for their hepatorenal protection and cytotoxic effects^[16]. Despite of the limited antimicrobial activity of extracts, it is noteworthy that *P. aeruginosa* and *C. albicans* were the most susceptible microorganisms. Particularly, both organisms are frequently coexisting opportunistic pathogens, forming microbial biofilms that can lead to severe illness and serious threat to human health^[33].

To the best of our knowledge, this is the first study to investigate the possible antimalarial effect of *A. lindleyi* extracts. Evaluating the extracts and compounds revealed promising antiplasmodial activity of ethanol extract and some components. Moreover, this plant can be useful as prophylactic against *C. albicans* and *P. aeruginosa*. The identified compounds were reported for the first time from this species excluding compounds 7 and 8.

Conflict of interest statement

We declare that we have no conflict of interest.

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