Powerful Antioxidant and Pro-Oxidant Properties of *Cassia roxburghii* DC. Leaves Cultivated in Egypt in Relation to Their Anti-Infectious Activities

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Leaf extracts of Cassia roxburghii *DC., prepared in petroleum ether, chloroform, ethyl acetate, butanol, and methanol/water (70:30, v/v), were evaluated as antioxidant, pro-oxidant, anti-infectious, and cytotoxic agents. The major metabolite of each extract was identified by chromatographic and spectroscopic means. The redox properties were assessed with a battery of assays, which revealed that the ethyl acetate extract demonstrated an interesting scavenging activity of DPPH and superoxide radicals and an ascorbic acid-like pro-oxidant activity. All the tested extracts showed moderate antiplasmodial activity against a chloroquine-resistant strain of* Plasmodium falciparum*, by possible disruption of parasite fine redox balance. Cytotoxicity was evaluated against a human breast cancer cell line. The antimicrobial activities of the extracts were estimated against representative bacterial strains (*Staphylococcus aureus, Enterococcus hirae, Pseudomonas aeruginosa, Escherichia coli*) and fungal species (*Candida albicans, Aspergillus niger*). The ethylacetate extract possessed the highest redox properties and*

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exhibited the highest antiplasmodial activity; there was no correlation between antibacterial activity and the redox properties of the extracts.

KEYWORDS Antimicrobial, antiplasmodial, Cassia marginata *Roxb, radical scavenging*

INTRODUCTION

Plants belonging to *Cassia* species are extensively used in traditional medicine in various parts of the world against a wide range of ailments (13). Various species of *Cassia* are reported to have antiviral (1), anti-inflammatory (6), larvicidal (7), antiprotozoal (22), antimalarial (14, 28), hepatoprotective (19), and anticancer (24) properties.

Cassia roxburghii DC. (Caesalpiniaceae), also known as *C. marginata* Roxb. (26), is a small deciduous tree native to Sri Lanka, southern India, and Egypt, where it is used in folk medicine to treat various liver disorders. The *in vivo* hepatoprotective effect of *Cassia roxburghii* seed have been reported (2). Skin diseases such as ringworm infections and the wound healing potential of *Cassia roxburghii* seed and leaf extracts have been reported (21). It has also been used as a cathartic agent (11). Phytochemical studies have demonstrated the presence of alkaloids, sterols, anthraquinones, glycosides, tannins, and flavonoids in *C. marginata* (5, 13, 20). In spite of this phytochemical richness, little is known about the redox capacity and bioactive properties of *C. marginata*.

The aim of this study was to evaluate the redox properties of *Cassia roxburghii* leaves. The *in vitro* anti-infectious activity of leaf extracts was evaluated against representative Gram-positive and Gram-negative bacterial strains (*S. aureus* CIP 4.83, *E. hirae* CIP 5855, *P. aeruginosa* CIP 82118, *E. coli* CIP 53126), fungal species (*C. albicans* IP 48.72, *A. niger* IP 1431.83), and a protozoan (*Plasmodium falciparum*) in relation to the redox capacity of the extracts. Cytotoxicity against a human breast cancer cell line (MCF-7) is also reported.

MATERIALS AND METHODS

Plant Material

Cassia marginata Roxb (*C. roxburghii* DC.) leaves were collected in December 2006 from the Orman Botanical Garden, Giza, Egypt. Plant samples were authenticated by the taxonomist of the Orman Botanical Garden. A voucher specimen No. C-5-63 was kept in the Herbarium of Orman Garden.

Extraction and Isolation

The shade dried powdered leaves of *C. roxburghii* (250 g) were defatted with petroleum ether. The crude petroleum ether extract (I), 17 g, was chromatographed on a flash column of silica gel G, eluted with petroleum ether (60–80) with gradient increase of polarity with ethyl acetate (0.5, 1, 2, 3, and 5%), allowing the isolation of emodin from fraction eluted with 2% ethyl acetate. The defatted powder was then extracted with methanol/water (70:30, v/v) and evaporated under vacuum yielding the total defatted extract (V), 60 g, which was further fractionated with chloroform (II), 6 g; ethyl acetate (III), 15 g; and butanol (IV), 12 g. Part of the total extract (V) was kept for biological study. The chloroform fraction (II) was applied to a silica gel column, and eluted with n-hexane/ethyl acetate with increasing polarity, resulting in the isolation of aloe-emodin. The ethyl acetate fraction (III) was subjected to reversed phase liquid chromatography on Sephadex LH20 to give quercitrin. The butanol fraction (IV) was fractionated on polyamide to yield three fractions that were purified by paper chromatography (15% AcOH), and then on a Sephadex LH20 sub-column to obtain emodin glucoside and aloe-emodin glucoside. Pure compounds were chromatographed with two-dimensional TLC on cellulose using butanol:acetic acid:water, 4:1:5 (BAW) and 15% acetic acid as solvent systems.

Chemicals

Dimethyformamide (DMF), dimethylsulfoxide (DMSO), acetonitrile (ACN), ethanol, linoleic acid, xanthine, xanthine oxidase, superoxide dismutase, ascorbic acid, α-tocopherol (vitamin E), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Tween 20, β-carotene, H_2O_2, $FeCl_3$, diethylenetriamine pentacetate (DTPA), 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) were purchased from Sigma (Sigma-Aldrich, France). Polyamide 0.05–0.16 mm (Roth) was used for column chromatography. TLC analysis was carried out using cellulose and silica gel 60 F254 plates (Merck).

Instruments

UV absorption spectra were recorded on UV-visible double beam UWD-3500 spectrophotometer (Labomed, Inc.). Mass spectrometry analyses were performed on a Finnigan MAT 112 electron impact ionization at 70 eV. ^{1}H and ^{13}C NMR spectra were done on a Varian Mercury VX 300 NMR spectrometer at 300 MHz and 75 MHz, respectively, using DMSO-d_6 or $CDCl_3$ as solvents. The chemical shifts were reported in ppm values using tetramethylsilane (TMS) as the internal standard. Chromatograms were visualized under UV light at λ

366 nm before and after spraying with ammonia (Naturstoff reagent A, Roth) reagent. Electron paramagnetic resonance (EPR) spectra were obtained at X-band on a Bruker EMX-8/2.7 spectrometer (Bruker, Wissembourg, France) (9.86 GHz) equipped with a high-sensitivity cavity (4119/HS 0205) and a gaussmeter (Bruker). A flat quartz cell FZK160- 5 × 0.3 mm (Magnettech, Berlin, Germany) was used for analysis. EPR data processing and spectrum computer simulation were performed using WINEPR and SIMFONIA software (Bruker). Typical scanning parameters were: scan rate, 1.2 G/s; scan number, 1; modulation amplitude, 1 G; modulation frequency, 100 kHz; microwave power, 20 mW; sweep width, 100 G; sweep time, 83.88 s; time constant, 40.96 ms; magnetic field, 3450–3580 G.

Major Chemical Components in Tested Fractions

Emodin was obtained from the petroleum ether fraction (I). The structural identification was carried out by UV, MS, and NMR spectroscopies. The results match with the previously reported data of emodin (8).

Aloe-emodin was obtained from the chloroform extract (II). The anthraquinone was isolated as yellowish needles from methanol, mp 220–222°C. EIMS, m/z: 270 [M^+]; ^{1}H NMR ($CDCl_3$,300 MHz): δ (ppm) 12.04 (s, 1H, OH-8), 11.97 (s, 1H, OH-1), 7.76 (dd, J = 0.76 and 7.52Hz,1H, H-5), 7.60 (d, J = 8.1 Hz, 1H, H-6), 7.59 (d, J = 0.4 Hz, 1H, H-4), 7.23 (dd, J = 0.74 and 8.4 Hz, 1H, H-7), 7.04 (d, J = 0.4 Hz, 1H, H-2), 4.50 (s, 2H, H-3).

Quercitrin (quercetin 3-*O*-α-rhamnoside) was obtained from the ethyl acetate extract (III). The structural identification of quercetin-3-*O*-α-rhamnopyranoside was carried out by UV, MS, and NMR spectroscopy. UV λ_{max} (MeOH): 265, 298sh, 351 nm; (+NaOMe): 271, 326sh, 398 nm; (+$AlCl_3$): 275, 304, 330, 422 nm; (+$AlCl_3$/+HCl): 269, 300sh, 353, 398 nm; (+NaOAc): 269, 317, 362 nm; (+NaOAc /H_3BO_3): 259, 299, 378 nm. EIMS, m/z: 448 [M^+], 302 [M^+ - rhamnose unit]. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 12.60 (s, 1H, 5-OH), 7.29 (d,J = 1.5 Hz, 1H, H-2′), 7.24 (d, J = 8.4 Hz, 1H, H-6′), 6.85 (d, J = 8.4 Hz, 1H, H-5′), 6.36 (br.s,1H, H-8), 6.19 (br.s, 1H, H-6), 5.25 (s, 1H, H-1″), 0.81 (d, J = 6.0 Hz, 3H,–CH_3). ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) 156.9 (C-2), 134.6 (C-3), 178.2 (C-4), 161.8 (C-5), 99.3 (C-6), 165.1 (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′), 102.3 (C-1″), 70.8 (C-2″), 71.0 (C-3″), 71.7 (C-4″),70.5 (C-5″), 18.0 (C-6″). The MS, ^{1}H NMR, and ^{13}C NMR data are in agreement with those of quercetin-3-*O*-α- rhamnpyranoside (9).

Two pure anthraquinone glycosides from the butanol extract (IV) were chromatographed with two-dimensional TLC on cellulose (BAW/15% acetic acid as solvent systems). The two anthraquinone glycosides had R_f values of 0.34 and 0.43 in BAW and 0.70 and 0.62 in 15% acetic acid, respectively. The two compounds were identified by chemical tests (positive

Borntrager's test and positive Molish's test), acid hydrolysis with HCl (2 N), and co-chromatography with authentic standards (BAW 4:1:5 as the solvent system). This enabled glucose to be identified as the sugar moiety and emodin and aloe-emodin as the aglycone. ESI mass $[M+2H]^+$ 596. The two compounds were identified as emodin diglucoside and aloe-emodin diglucoside.

Redox Properties

Simple pro-oxidant assay

Extract (100 mg/L) and DMPO (50 mM) were diluted in DMSO/HEPES buffer, pH = 7.4, (10/90) solution. EPR spectra were recorded (accumulation of 10 scans) every 15 minutes during 1 h. Ascorbic acid (100 μM) and artemisinin (100 μM) were used as positive and negative controls, respectively.

Fe^{3+}/H_2O_2 pro-oxidant assay

HEPES buffered solutions (50 mM, pH = 7.4) containing 250 μM $FeCl_3$, 250 μM hydrogen peroxide, 100 mg/L of extract in DMF (100 μL), and 50 mM DMPO were incubated for 2 min at room temperature prior to EPR measurements (accumulation of 10 scans). Ascorbic acid (100 μM) and artemisinin (100 μM) were used as positive and negative controls respectively.

Fe^{2+} pro-oxidant assay

Solutions containing 8.3 mM $Fe(NH_4)_2(SO_4)_2$, 2.2 mg/mL of extract in DMF, and 167 mM DMPO were incubated for 10 min at room temperature prior to EPR spectra recording (accumulation of 10 scans). As a positive control, artemisinin (6.67 mM) replaced the extract (15).

DPPH Assay

The antioxidant activity was evaluated using the stable 2,2-diphenyl-2-picrylhydrazyl nitrogen-centered free radical ($DPPH^•$). $DPPH^•$ in ethanol (300 μM, 100 μL) was added to 200 μL of the test compounds at different concentrations in the appropriate solvent (MeOH, EtOH, $MeOH/H_2O$ 2:1, v/v). Each mixture was then mixed thoroughly and the absorbance was recorded every 5 min for 30 min, using a microplate reader (UVMax, Molecular Devices). The decrease in $DPPH^•$ absorbance (at λ 530 nm) was monitored. $DPPH^•$ solution (100 μL) in the respective solvent (200 μL) served as the blank. All tests were performed in triplicate. The radical scavenging activity of the

samples (antioxidant activity) was expressed in terms of IC_{50} (concentration in mg/L required for a 50% decrease in $DPPH^{\bullet}$ absorbance).

A plot of absorbance vs. concentration was made to establish the standard curve and to calculate the IC_{50}. Ascorbic acid and Trolox (water soluble form of vitamin E) were used as positive controls and vitamin E acetate was used as the negative control.

Beta-Carotene Bleaching Assay

The antioxidant activity of extracts was determined according to the β-carotene bleaching method (12, 16, 23) with the following modifications: A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 1 mL of chloroform. 500 μL of this solution was then transferred into a round-bottom rotary flask containing 15 mg of linoleic acid and 200 mg of Tween 20. After removing the chloroform under a nitrogen stream, 50 mL of aerated distilled water was added to the flask with manual shaking. Aliquots of 5 mL of this prepared emulsion were transferred into tubes containing 200 μL of extracts or α tocopherol (50 mg/L), which was used as positive control. The blank consisted of 0.2 mL of the respective solvent without the extract. Zero time absorbance was recorded at λ 470 nm as quickly as possible. The samples were then subjected to thermal autoxidation at 50 °C in a temperature-controlled eight-cell holder. Subsequent absorbance readings were recorded at regular time intervals until the color of the β-carotene in the control sample had disappeared (25 min). The extent of inhibition of β-carotene bleaching is related to the concentration of antioxidant compounds. All samples were assessed in triplicate. Antioxidant activity (AA) was calculated as percentage inhibition of β-carotene bleaching relative to the blank using the following equation:

$$AA = [1 - (A_i - A_t)/(A'_i - A'_t)] \times 100$$

where A_i is the measured absorbance value of the sample at zero time; A_t is the measured absorbance value of the sample after incubation (25 min) at 50 °C; A'_i is the measured absorbance value of the blank at zero time, and A'_t is the measured absorbance value of the blank after incubation (25 min) at 50 °C.

Superoxide Assay

The superoxide radical was generated by reaction of the xanthine–xanthine oxidase system. The extract sample was dissolved in dimethyl sulfoxide (DMSO). 0.5 mM xanthine (80 μL), DMSO, or extract solution (final volume 32 μL), 1 M DMPO (32 μL), appropriate volume of HEPES buffer containing

0.1 mM DTPA, and xanthine oxidase (20 units/mL) (10 μL) were mixed in a test tube to give a total volume of 400 μL. The mixture was quickly transferred to a flat quartz cell. The EPR spectra of the DMPO-OOH spin adducts were recorded 90 s after xanthine oxidase addition. The radical scavenging activity of the samples was expressed in terms of percent inhibition of DMPO-OOH spin adduct according to the following formula:

$$\%\,\text{Inhibition} = [(I_{blank} - I_{test})/I_{blank}] \times 100)$$

where I_{blank} is the intensity of the first peak of the blank spectrum, I_{test} is the intensity of the first peak of the test sample spectrum. Ascorbic acid was used as positive control.

Antimicrobial Assays

The extracts were dissolved in water/DMSO to obtain an initial concentration of 1000 μg/mL. The resulting solutions were then diluted in microtiter plates in culture medium: trypcase soja (Biomérieux, Craponne, France) for bacteria and Sabouraud (Biomérieux) for fungi and yeast. Gram-positive strains *S. aureus* CIP 4.83 and *E. hirae* CIP 58.55 and Gram-negative strains, *P. aeruginosa* CIP 82118 and *E. coli* CIP 53126 were used for the antimicrobial assays. For the antifungal assay cultures,a yeast, *C. albicans* IP 48.72, and mold, *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 10^6 cells/mL and 10^5 spores/mL for bacteria and fungi, respectively. Minimal inhibitory concentrations (MICs) and minimal germicidal concentrations (MBC or MFC) were determined after incubation of the bacterial strains at 37 °C for 24 h and the fungal strains at 30 or 22.5 °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 were determined by subcultivating on corresponding agar plates after incubation of the bacterial strains at 37 °C for 24 h and fungal strains at 30 or 22.5 °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 upon 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.

Antiplasmodial Assay

Antiplasmodial activity was tested according to the protocol previously reported (18). RPMI 1640 medium [BioWhittaker, Cambrex (cat no. BE12-702F), Belgium] containing L-glutamine (BioWhittaker, cat no. BE17-605E), 25 mM HEPES (BioWhittaker, cat no. 17-737F), and 10% human serum (Etablissement Français du Sang, EFS, Toulouse, France), was used to cultivate *P. falciparum (P.f.)*. Human red blood cells (RBCs; group O±) were obtained from EFS. They were extensively washed with RPMI medium to remove plasma and leucocytes. Leucocyte-free erythrocytes were stored at 50% hematocrit for a maximum period of 21 days. *P.f.* asexual blood stage parasites were propagated by incubation at 37 °C in *P.f.* culture media at 3–5% hematocrit in a controlled atmosphere (5% CO_2, 100% relative humidity). Parasitized RBCs were maintained in 25 cm^2 culture flasks (TPP, Switzerland, ref 90025). The reference drugs chloroquine (CQ) and artesunate were obtained from Sigma (ref C6628) and Cambrex, respectively. CQ was dissolved in culture medium and artesunate in ethanol (stock solutions: 10 mg/mL) and stored at –20 °C prior to testing. For extract assays, serial extract dilutions were made in *P.f.* culture media and added to 96-well (TPP) culture plates. All samples were tested in triplicate. *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% of CO_2. [^{3}H]-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 [^{3}H]-hypoxanthine was determined with a beta-counter (1450-Microbeta Trilux, Wallac-PerkinElmer). Growth-inhibition percentages were plotted as a semi-logarithmic function of drug concentration. The IC_{50} values were determined by linear regression analysis on the linear segments of the curves. Assays were repeated three times. Controls were carried out to assess the background (negative control) and parasite growth (positive control).

Cytotoxicity Assay

The cytotoxicity was tested according to the protocol previously reported (18). Cytotoxicity was estimated on human breast cancer cells (MCF7) cultured in the same conditions as those used for *P. falciparum*, except that 10% human serum was replaced by 10% fetal calf serum (Cambrex). After trypsinization, cells were distributed in 96-well plates at 2×10^4 cells/well in 100 µL of culture medium added to 100 µL of the same medium containing the test samples at various concentrations (the final concentrations in the wells were 1, 10, and 100 µg/mL). Cell growth was estimated by a colorimetric assay based on sodium 2,3-bis(2-methoxy-4-

nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt (XTT; Sigma, Saint Quentin Fallavier, France) reduction. After 48 h of contact between cells and test compounds the culture medium was replaced by 50 µl of a XTT in water solution (0.5 mg/mL), and cells were incubated for 180 min. XTT was converted into a formazan product, detected at λ 450 nm. IC_{50} values were determined graphically from dose–response curves (the positive control being doxorubicin; Sigma). Experiments were performed twice in triplicate.

RESULTS

The main flavonoid or anthranoids present in each extract after purification and identification were emodin, aloe-emodin, and quercetin-3-*O*-α-rhamnpyranoside in petroleum ether (I), chloroform (II), and ethyl acetate (III) extracts, respectively, and emodin diglucoside and aloemodin diglucoside in butanol extract (IV). The four extracts, along with the defatted methanol/water (total extract) (V) of *C. roxburghii* DC. leaf, were evaluated for their redox molecular pool with different pro-oxidant and antioxidant assays.

To explore whether the extracts possess artemisinin-like and/or an ascorbic acid–like pro-oxidant activities or not, the pro-oxidant capacity of *Cassia roxburghii* DC. leaf extracts was evaluated using EPR spectroscopy according to three different assays. The results are summarized in Table 1. The ethyl acetate extract (III) demonstrated an ascorbic acid-like pro-oxidant activity.

The antioxidant properties were assessed using DPPH, β-carotene-bleaching, and superoxide assays (Table 2). The antioxidant activity of the extracts was expressed as IC_{50} and percentage inhibition. The ethyl acetate extract (III) demonstrated the highest antioxidant properties in the DPPH and superoxide radical scavenging assays.

The antimicrobial potential of *Cassia roxburghii* DC. leaf extracts was evaluated on selected bacterial and fungal strains. Table 3 demonstrates the antimicrobial properties of the extracts. The majority of the extracts exhibited antibacterial activity against Gram-positive bacteria, with MIC values around 125 µg/mL. Interestingly, the Gram-negative strain *P. aeruginosa* and the yeast *C. albicans* were more susceptible to the majority of the extracts, with MIC values of 62.5 µg/mL.

Cassia roxburghii DC. leaf extracts were assessed for their antiplasmodial activity against a chloroquine-resistant strain of *P. falciparum*. The cytotoxicity of the extracts was also evaluated on a human breast cancer cell line (MCF-7). Selectivity index was calculated as the ratio of CC_{50} value in MCF-7 cells to IC_{50} value in the CQ-resistant *P.f*. strain FcB1. The results are shown in Table 4.

TABLE 1 Pro-oxidant capacity of *Cassia roxburghii* DC leaf extracts

Extract	Simple pro-oxidant assay	Fe^{3+}/H_2O_2 pro-oxidant assay $I_1 \pm$ SD (RSD %)	Fe^{2+} pro-oxidant assay $I_1 \pm$ SD (RSD %)
I	Inactive	$I_1 = 7317 \pm 327$ (4 %) quadruplet[c]	Inactive
II	Inactive	$I_1 = 6767 \pm 510$ (8 %) quadruplet[c]	$I_1 = 517 \pm 74$ (14 %) sextuplet[d]
III	Inactive	$I_1 = 9967 \pm 332$ (3 %) quadruplet[c]	$I_1 = 1857 \pm 23$ (1 %) sextuplet[d]
IV	Inactive	$I_1 = 8867 \pm 527$ (6 %) quadruplet[c]	$I_1 = 1648 \pm 139$ (8 %) sextuplet[d]
V	Inactive	$I_1 = 8667 \pm 330$ (4 %) quadruplet[c]	$I_1 = 2057 \pm 29$ (1 %) sextuplet[d]
artemisinin	Inactive	Inactive	$I_1 = 6133 \pm 464$ (8 %) sextuplet[d]
ascorbic acid	I_1[a] = 1534 doublet[b] (ascorbyl radical)	$I_1 = 13137$ doublet[b] (ascorbyl radical)	$I_1 = 783 \pm 73$ (9 %) doublet[b] (ascorbyl radical)

[a]I1: intensity of the first peak of the EPR signal.
[b]aH = 1.8 Gauss; g = 2.0051.
[c]aN = 14.7 Gauss; aH = 14.5 Gauss; g = 2.0058.
[d]aN = 15.2 Gauss; aH = 21.1 Gauss; g = 2.0053.

TABLE 2 Antioxidant Capacity of *Cassia roxburghii* DC. Leaf Extracts

	DPPH assay		β-Carotene bleaching assay	Superoxide assay
Extract	Solvent	IC_{50}[a] (mg/L)	Percentage inhibition[b] ± SD	Percentage inhibition[c] ± SD
I	EtOH	173.81 ± 4.94	21.39 ± 0.58%	Inactive
II	EtOH	90.19 ± 2.02	28.50 ± 3.29%	30.08 ± 2.49%
III	EtOH	18.36 ± 0.09	33.85 ± 6.31%	75.00 ± 1.16%
IV	ACN/H_2O	88.00 ± 3.30	34.82 ± 0.97%	53.03 ± 0.62%
V	ACN/H_2O	69.05 ± 0.74	39.72 ± 2.77%	59.38 ± 2.77%
Trolox	EtOH	8.36 ± 0.05	76.35 ± 0.39%	ND[d]
Vitamin C	ACN/H_2O	3.81 ± 0.08	Inactive	100%[e]

[a]Concentration required for a 50% decrease in DPPH• absorbance.
[b]% inhibition of the β-carotene bleaching using a 100 mg/L extract solution.
[c]% inhibition of the EPR signal intensity of the reference superoxide radical using a 50 mg/L extract solution.
[d]Not determined.
[e]% inhibition of the EPR signal intensity of the reference superoxide radical using a 10 mg/L ascorbic acid solution.

DISCUSSION

This study allowed identification of two anthraquinones, emodin and aloe-emodin, and two anthraquinone glycosides, emodin diglucoside and aloe-emodin diglucoside, besides quercitrin from *C. roxburghii* leaves, which

TABLE 3 Antibacterial and Antifungal Activity of *Cassia roxburghii* DC. Leaf Extracts (all concentrations expressed in μg/mL)

	S. aureus		*P. aeruginosa*		*E. hirae*		*E. coli*		*A. niger*		*C. albicans*	
Extract	*MIC*[a]	*MBC*[b]	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MFC*[c]	*MIC*	*MFC*
I	125	250	62.5	125	125	500	125	500	125	–	62.5	125
II	125	>500	>500	>500	250	> 500	500	>500	500	>500	> 500	>500
III	125	>500	>500	>500	>500	>500	>500	>500	>500	> 500	>500	>500
IV	125	500	62.5	250	125	>500	125	500	125	–	62.5	125
V	125	500	62.5	125	125	>500	125	500	125	–	62.5	125
N.A.[e]	125	125	> 125	> 125	6.25	6.25	3.125	3.125	–	–	–	–
BL[f]	> 125	> 125	> 125	> 125	> 125	> 125	> 125	> 125	3.125	–	125	> 125
CL[g]	> 125	> 125	> 125	> 125	> 125	> 125	> 125	> 125	1.562	–	3.125	6.25

[a]MIC: minimum inhibitory concentration (μg/mL).
[b]MBC: minimum bactericidal concentration (μg/mL).
[c]MFC: minimum fungicidal concentration (μg/mL).
[d]NA: nalidixic acid.
[e]BL: bifonazole.
[f]CL: clotrimazole.

TABLE 4 Antiplasmodial Activity and Cytotoxicity of *Cassia roxburghii* DC. Leaf Extracts

Extract	IC_{50} ± SD (μg/mL) FcB1 strain	CC_{50} ± SD (μg/mL)[a] MCF-7	Selectivity index
I	28.5 ± 5.5	31 ± 0	1.1
II	19.5 ± 1.5	44 ± 6	2.26
III	17.25 ± 3	53 ± 6.5	3.1
IV	20 ± 0.5	48.5 ± 6.5	2.43
V	25 ± 2	29 ± 0	1.2
CQ	0.072 ± 0.015 (n = 5)	9.7	134.0
Sodium artesunate	0.0024 ± 0.0012	3.97[b]	1654.0

[a]CC_{50} (μg/mL): drug concentration needed to cause 50% decrease of the cellular viability.
[b]Ref. (18).

have been previously reported in roots and stem bark (19) and in other *Cassia* species such as *C. fistula, C. nigricans*, and *C. tora* (3, 10).

The redox properties of the five extracts were evaluated with six assays, two based on UV-visible absorbance measurements and four on radical species detection by EPR spectroscopy. These different assays were used to compare the redox molecular content of the extracts by analyzing two facets of the molecular redox properties: the pro-oxidant and the antioxidant capacities. Some natural products are pro-oxidant and cannot be antioxidant (e.g., artemisinin). Other compounds may undergo redox cycles and present both properties (pro-oxidant and antioxidant) depending on conditions, such as quinones, pseudo-quinones, and their hydrogen-conjugates (i.e., ascorbic acid).

Table 1 compares the pro-oxidant capacities of the extracts. None of the extracts may produce free radicals by direct oxidation in solution as ascorbic acid does. In the presence of Fe^{3+} and H_2O_2, all the extracts generated free radicals as ascorbic acid does while artemisinin cannot. These results are in agreement with the presence of quinoid or pseudo-quinoid compounds, some of which were identified in this study. In the presence of Fe^{2+}, extracts II–V generate radical species in amounts comparable to those generated by ascorbic acid and much less than that generated by artemisinin.

Altogether, these results show that the extracts do not contain peroxide-like molecules and contain pseudo-quinones or quinone-like molecules that generate free radical species when introduced in a redox cycle. The highest ascorbic acid-like pro-oxidant activity was observed in extract III. It must be noted that extract I showed no activity in two pro-oxidant assays.

By examining the results in Table 2, which compares the antioxidant properties, it appears that extract III is the most active. It demonstrates the highest activity in the DPPH and superoxide assays. In the β-carotene bleaching assay, extract V was slightly more active, which, since this assay discriminates hydrophilic and lipophilic antioxidants, indicates a higher content of lipophilic redox molecules. Extract I was the least active. Emodin,

aloe-emodin, and quercitrin (the major components of extract I, II, and III, respectively) have been reported to exhibit free radical scavenging and antioxidant roles (25, 27). Certainly, these compounds are responsible for the interesting antioxidant properties of *C. roxburghii* leaves, but not alone. The isolated pure compounds showed less antioxidant activity than the tested extracts in the DPPH assay (unpublished results). Other compounds may exert synergistic action.

Altogether, the pro-oxidant/antioxidant capacity of the five extracts shows that extract III has the highest redox molecular content, which can be related to the antiplasmodial properties. All the tested extracts showed moderate antiplasmodial activity against a chloroquine-resistant strain of *P. falciparum* with IC_{50} values ranging from 17.25 to 28.5 μg/mL. The ethyl acetate extract (III) gave the highest antiplasmodial activity. The selectivity indices were calculated after the evaluation of cytotoxicity on a human breast cancer cell line. The most selective was ethyl acetate extract.

These results suggest a relationship between the redox capacity and the antiplasmodial activity of the extracts. The redox properties may be responsible, at least in part, for the observed antiplasmodial activity of *C. roxburghii* leaves. Oxidative stress has been reported to be a key clinical and pathobiochemical factor as well as an effective therapeutic approach in malaria. Plasmodium creates a fine balance of redox interactions in parasitized red blood cells (4). Disruption of this redox equilibrium by *Cassia* redox active extracts may be unfavorable for parasite development.

Extracts I, IV, and V possessed antimicrobial activity with MIC values ranging from 62.5 to 125 μg/mL. Of special interest is the antibacterial activity against the Gram-negative strain *P. aeruginosa,* which was more susceptible to these extracts (MIC = 62.5 μg/mL) than the Gram-positive strains used in the assay. The extracts exhibited better antifungal properties against *C. albicans* than *A. niger* (Table 3). This antimicrobial activity can support the traditional use of the plant in skin infections and in wounds.

The antimicrobial activity of *C. roxburghii* leaf extracts did not correlate with their redox properties. This may suggest that the redox status is not as critical for microbial growth as it is for plasmodium.

CONCLUSION

Cassia roxburghii leaf extracts possess interesting redox capacity (antioxidant and ascorbic acid-like pro-oxidant activities), moderate antiplasmodial properties, and antimicrobial (antibacterial and antifungal) effects. A direct relation between the interesting redox power and the antiplasmodial properties deserves further evaluation of the role of *C. roxburghii* leaf extracts in combating oxidative stress induced pathogenesis. The antimicrobial properties described do not correlate with these redox

properties, suggesting that redox interactions are not the major factor in the antimicrobial activity.

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