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Antibacterial, cytotoxic, and schistosomicidal activities of the methanolic extract from *Cassia grandis* L.f. (Fabaceae) stem bark and its fractions

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***Cassia grandis* L. (Fabaceae), a native tree from Amazon Forest, has been used in folk medicine against worms and intestinal parasites, and to treat stomach and respiratory problems, blood diseases, among others uses. This study aimed to evaluate the antioxidant, cytotoxic, antimicrobial, and schistosomicidal activities of the methanolic extract from *C. grandis* stem bark (CgME) and to elucidate the chemical profile of its active fractions. The antioxidant activity of *C. grandis* stem bark methanolic extract (CgME) and its fractions were determined by DPPH radical scavenging assay and by the total phenolics and flavonoid contents. The antimicrobial activity was performed by microdilution. The cytotoxicity against MCF-7 (breast), NCI-H292 (lung), and HL-60 (leukemia) cancer cell lines was evaluated by the MTT method. The schistosomicidal activity was investigated *in vitro* against adult *Schistosoma mansoni* couple worms. The phytochemical profile of the active fractions was determined by GC-MS and UPLC-MS. The hexane fraction from the CgME was cytotoxic to NCI-H292 and HL-60 cancer cell lines and both major compounds clionasterol and lupeol acetate, determined by GC-MS, are well known for their cytotoxicity against cancer cells. The ethyl acetate fraction (CgEF) exhibited both antibacterial, against multidrug-resistant *S. aureus*, and schistosomicidal activities, which could be attributed to the presence of flavonoids, such as catechin derivatives, quercetin, and luteolin in the CgEF. These results agree with the popular uses of *C. grandis* and should stimulate future research on this species.**

Key words: Antimicrobial activity, antioxidant, *Staphylococcus aureus*, clinical isolates, *Schistosoma mansoni*, cytotoxicity.

INTRODUCTION

The use of medicinal plants as a therapeutic resource in the cure and prevention of diseases is as old as the human culture. This ancient knowledge has been used in the research and development of modern medicines.

Currently, up to 35% of the commercially available medicines are originated from or inspired by natural products (Calixto, 2019). Plants are a limitless source of bioactive compounds potentially useful to treat many human or animal disorders (Guidoti et al., 2019). A broad investigation of their biological activities is the main step in development of effective alternative medications (Singh et al., 2017).

Fabaceae (Leguminosae) is one of the largest Angiosperm families with approximately 730 genera and almost 20,000 species from small herbs to high trees widespread around the world. In Brazil, Fabaceae is the largest plant family with more than 2,700 species sorted among 212 genera (Andrade et al., 2009; Forzza et al., 2010; Lima et al., 2015). *Cassia* species are distributed in tropical and subtropical regions of America, Africa, and Asia. Besides their uses in folk medicine as purgative, many other pharmacological activities have been reported, such as antibacterial, antifungal, antimalarial, anti-inflammatory, and hepatoprotective (Viegas Junior et al., 2006).

Cassia grandis L., commonly known as *pink cassia*, is native from the Brazilian Amazon forest and nowadays very common all-around Brazil due to its use in urban landscaping. This species is a large tree that can reach 12 m in height. It has small deciduous leaves, pinkish-yellow flowers and pod fruits that can weigh up to a kilo (Joshi and Kapoor, 2003). *C. grandis* is considered a multipurpose plant due to its many uses. As a medicinal plant, it is used against worms and intestinal parasites, to treat stomach and respiratory problems, infected wounds, blood diseases, insulin resistance, among others (Parra and Sardiñas, 2000; Lodha et al., 2010; Meena et al., 2009). Phytochemical studies have shown the presence of alkaloids, phytosterols, saponins, flavonoids, tannins, and anthraquinones in different organs of the plant (Meena et al., 2010).

Based on its medicinal uses, this study aimed to evaluate *in vitro* the antioxidant, cytotoxic, antimicrobial, and schistosomicidal activities of *C. grandis* stem bark methanolic extract and to determine the phytochemical profile of its active fractions by GC-MS and UPLC-MS.

MATERIALS AND METHODS

Plant material and extraction

The stem bark of *C. grandis* L.f. (Fabaceae) was collected inside the campus of the Federal University of Pernambuco, Recife, Brazil (8°02'47.4"S 34°57'03.2"W, sea level). Recife has tropical weather, with temperatures between 20-30ºC during all year, a rainfall season between April and August, and annual precipitation at about 2000 mm. A voucher specimen was identified by O. Cano at the IPA Herbarium and deposited under nº 89,340.

The plant material was dried at room temperature for seven days and powdered in a knife-mill (Willey type). The powder (2.6 kg) was extracted with methanol for 2 h under mechanical agitation. The solvent phase was filtered, and the procedure repeated twice. Thereafter, the organic phases were pulled together and evaporated to dryness at 40ºC under reduced pressure yielding 12.1% (CgME). The extract (50 g) was fractionated by solid-liquid partition with hexane and ethyl acetate to give the respective fractions: CgHF and CgEF, which yielded 1.5 and 18.5%, respectively. The remaining extract was called CgMF (79.9%).

Thin-layer chromatography analysis

A phytochemical screening was performed by TLC to determine the presence of the following classes of secondary metabolites: flavonoids, phenylpropanoids, terpenes and steroids, coumarins, quinones, alkaloids, and proanthocyanidins as described in Table S1 (Supplementary material).

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu's reagent (McDonald et al., 2001). Briefly, aliquots of 0.5 mL of the extract/fractions (1 mg/mL) and 0.1 mL of Folin-Ciocalteu's reagent (0.5 N) were mixed and incubated at room temperature for 15 min. After this period, 2.5 mL of saturated sodium carbonate solution was added and further incubated for another 30 min at room temperature. Thereafter the absorbance was measured at 760 nm. The total phenolic content of the extract/fractions were expressed as gallic acid equivalent (mg GAE/g of extract/fraction) based on a standard curve of gallic acid (1.56 to 100 µg/mL).

Determination of flavonoid content

The amount of flavonoid in the extract/fraction was determined by the $AlCl_3$ colorimetric method (Woisky and Salatino, 1998). Shortly, 500 µL of the extract/fractions (1 mg/mL) and 500 µL of $AlCl_3$ (2%) were mixed and incubated at room temperature for 60 min. Then, the absorbance was measured at 420 nm. The flavonoid content of the extract/fractions was expressed in as quercetin equivalent (mg QE/g of extract/fraction) based on a standard curve of quercetin (1 to 100 µg/mL).

Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography and mass spectrometry (GC-MS) analysis was performed at the Technological Development Park of the Universidade Federal do Ceará using a GCMS QP 5050A (Shimadzu, Kyoto, Japan) equipped with a non-polar AW Scientific DB- 1 MS capillary column (50 m × 0.25 mm × 0.25 µm). The oven temperature was set at 70°C with an increase of 4°C/min until 230°C and then maintained for 15 min. The carrier gas was helium, with a constant flow rate of 1.7 mL/min. The temperature of the ionization source was maintained at 280°C, ionization energy at 70 eV, and ionization current at 0.7 kV. Mass spectra were recorded from 30 to 450 m/z. Individual components were identified by

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matching their 70 eV mass spectra with those of the spectrometer database by using the Wiley L-Built library and by comparing their retention indices and fragmentation patterns with those of the NIST MS library (Stein et al., 1997) and those reported in the literature (Adams, 2001).

Ultra-performance liquid chromatography-mass spectrometry (UPLCMS) analysis

The chemical constituents of CgEF were analyzed on an Acquity H-Class UPLC (Waters®, MA, USA) equipped with a BEH C18 Column, 130 Å, 1.7 µm, 2.1 mm x 100 mm. The mobile phases used consisted of aqueous solution containing 2% MeOH, 5 mM ammonium formate, and 0.1% formic acid (eluent A), and MeOH containing 0.1% formic acid (eluent B), at a flow rate of 0.3 mL/min. Elution was performed in gradient mode and the initial condition (98% A / 2% B) was maintained for 0.25 s. The proportion of B was linearly increased to 99% in 8.5 min and then maintained for 60 s, followed by the immediate decrease to 2% B, which was maintained for 90 s. Ten microliters of sample were injected. The column temperature was maintained at 40°C and the injector at 10°C. The UPLC system was coupled to a single quadrupole mass spectrometer SQ Detector 2 (Waters®). The optimized conditions were as follows: capillary voltage 3.5 Kv; cone voltage 30 V; desolvation temperature 450°C; and a desolvation gas flow rate of 650 L/h. The data acquisition was done in full scan mode, searching for masses between 100 and 1000 Da, in negative ionization mode. The chromatograms and mass spectra were acquired with MassLynx™ software (Waters®).

Antioxidant activity

Total antioxidant activity

The total antioxidant activity of the extract/fractions was evaluated by the phosphomolybdenium method (Prieto et al., 1999). The assay is based on the reduction of Mo(VI) to Mo(V) by the extract/fractions and subsequent formation of a green phosphate/Mo(V) complex at acid pH. Each sample solution (0.3 mL) and ascorbic acid (100 mg/mL) was combined with 3 mL of reagent (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank solution was made with 3 mL of reagent solution and 0.3 mL of the solvent used for sample dilution. All tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After this period, the samples were cooled to room temperature, and the absorbance was measured at 695 nm. The experiment was performed in triplicate. Total antioxidant activity was expressed as equivalents of ascorbic acid.

DPPH free radical scavenging activity

The free radical scavenging activity was measured using DPPH (2,2-Diphenyl- 1-picrylhydrazyl) according to the method described by Blois (Blois, 1958). The reaction mixture consisted of 1.0 mL of DPPH in MeOH (0.3 mM) and 1.0 mL of the extract/fractions (1.56 - 50 µg/mL) diluted in MeOH. Thereafter, it was incubated in the dark for 10 min, after which the absorbance was measured at 517 nm. Gallic acid (1.56-50 µg/mL) was used as a positive control. The EC_{50} was calculated by linear regression.

Cytotoxicity evaluation

Cell lines and culture

The cell lines MCF-7 (human breast adenocarcinoma), NCI-H292 (human lung mucoepidermoid carcinoma), and HL-60 (human promyelocytic leukemia) were obtained from the Rio de Janeiro Cell Bank, Brazil. The cells were maintained in DMEM or RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco™, USA), 1% antibiotic solution (penicillin 10 kU/mL + streptomycin 10 mg/mL) and 1% L-glutamine 200 mM.

Cytotoxicity assay

MTT reduction assay was used to determine the cytotoxicity of the extract/fractions. Briefly, the cancer cells were plated in 96-well plates at 1 x 105 cells/mL for adherent cells (MCF-7 and NCI-H292) or 3 x 105 cells/mL for HL-60. The extract/fractions at 50 µg/mL) dissolved in DMEM or RPMI 1640 with 0.5% DMSO were added to each well. Then, cells were incubated for 72 h, after which 25 µL of MTT (5 mg/mL) was added. Three hours later, the supernatant was removed, and the formazan crystals were dissolved in 100 µL DMSO. The absorbance was measured at 570 nm in a microplate reader (Thermo, USA). For the extract/fractions that inhibited more than 70% of cell viability, the IC_{50} was determined using different concentrations (50 – 1.56 µg/mL). Doxorubicin (5-0.01 µg/mL) was used as a positive control (Mosmann, 1983).

Antimicrobial activity

Strains and growth conditions

The strains used in the disk diffusion test consisted of nine microorganisms: four Gram-positive bacteria *Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis*, and *Enterococcus faecalis*; three Gram-negative bacteria *Pseudomonas aeruginosa, Escherichia coli*, and *Serratia marcescens*; one alcohol-acid resistant bacteria *Mycobacterium smegmatis*; and one yeast *Candida albicans*. Three multidrug-resistant *S. aureus* clinical isolates from infected wounds were also used (Table S2 Supplementary material). All microorganisms were obtained from the Microorganism Collection of the Department of Antibiotics of the UFPE. Bacterial inocula were obtained from cultures with approximately 18-24 h of incubation at 35°C in Müller-Hinton agar for bacteria, and 24-48 h at 30°C in a Sabouraud agar medium. Microorganism suspensions were standardized according to the turbidity equivalent to 0.5 tube on the McFarland scale using a Genesys™ 10S spectrophotometer (Thermo Scientific, MA, USA), which corresponds to approximately 108 CFU/mL (CLSI, 2018).

Paper disc diffusion test

Whatman Nº 1 paper disks of 6 mm diameter were soaked with 20 µL of the extract/fractions (100 mg/mL) in DMSO, each disk having a final concentration of 0.4 mg of the extract/fractions. The disks were placed on the surface of solid medium previously seeded with the test microorganisms in Petri dishes. The dishes were incubated at 35°C for 24 h (bacteria) or 30°C for 48 h (yeast). The tests were performed in triplicate and the results were expressed in mm (Bauer et al., 1966). DMSO embedded disks were used as solvent control. As standard drugs, commercial kanamycin (30 µg) or ketoconazole (10 µg) disks were used.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC was determined by broth microdilution in round-bottom 96-well plates according to the CLSI M100 guideline (CLSI, 2018). Serial dilution of the extract/active fractions in Mueller Hinton broth (2000 – 3.91 µg/mL) was added to the wells containing an inoculum of 10^8

CFU/mL of the test microorganisms. The plates were incubated for 18 h at 35ºC, and then 20 µL resazurin (0.01%) was added to each well. MIC was determined as the concentration of the last well where there was no change from purple to pink. The content of the wells that remained purple was seed on Petri dishes containing Müller Hinton agar to determine the MBC, which is the concentration where there was no growth of colonies. All the analyses were performed in triplicate.

Schistosomicidal activity

Parasites and hosts

Schistosoma mansoni strain BH cercariae (Belo Horizonte, Brazil) were obtained from Keizo Asami Immunopathology Laboratory (LIKA) at UFPE. Thirty-day old *Swiss Webster* mice (n = 10), weighing 28 ± 2 g, were infected percutaneously with approximately 120 cercariae and maintained under standard conditions, with water and food *ad libitum*, in LIKA's animal facility. After 60 days, mice were euthanized, and adult worms were harvested through perfusion of the hepatic portal and mesenteric systems. The experimental protocol was approved by the Ethics Committee on Animal Experimentation of UFPE, under the nº 0013/2019.

In vitro assay

The adult worms couples harvested from mice were washed four times with RPMI 1640 medium supplemented with 20 mM HEPES, 100 µg/mL penicillin, 100 µg/mL streptomycin and 10% fetal bovine serum. Then, intact couples were transferred to 24-well culture plates containing 2 mL of the same medium (two worm couples/well) and incubated at 37ºC in a humidified atmosphere with 5% CO_2. After 2 h, the extract/fractions were added to each well from a stock solution of 1 mg/mL in 1.5% DMSO in RPMI 1640 to obtain concentrations of 50-400 µg/mL. Worm couples incubated with 1.5% DMSO in RPMI 1640 or praziquantel (PZQ) 3.12 µg/mL were used as negative and positive controls, respectively. All experiments were performed in triplicates and repeated twice. The worms were kept in culture for 3 days and monitored using an inverted optical microscope (Leica Microsystems, DM IL Wetzlar, Germany) every 24 h to evaluate the motor activity, oviposition, and mortality rate.

Motility and survival

The treatments were considered lethal when it was not possible to observe worms' movements for up to 2 min of observation. The motility of the worms was evaluated according to the viability criteria proposed by Horiuchi et al. (2005). Briefly, movements were scored in a 0-3 scale, being: Score 3- worms with typical movements, exhibiting peristalsis of the internal organs, suckers in movement, adhering to the bottom or sides of the culture plate; typical descriptions of worms of the negative control; Score 2- reduced movements throughout the body, peristalsis of internal organs and suckers; Score 1- movements only at the extremities or at only one of the extremities (anterior and/or posterior regions), with absence of peristalsis of the internal organs and not adhered suckers; Score 0- complete absence of motions and tegument with or without changes in coloration. The treatment was considered lethal when it was not possible to observe parasite movements for up to 2 min.

Cell viability assay of the S. mansoni couples

The cell viability of the *S. mansoni* couples after incubation with the extract/fractions or praziquantel was determined using MTT (Aires et al., 2014). Shortly, two worm couples were plated in each well using a 96-well culture plate containing 100 µL of MTT (5 mg/mL in PBS) and incubated at 37ºC for 30 min. Thereafter, MTT solution was replaced by 200 µL of DMSO to dissolve the formazan crystals. The absorbance was measured at 570 nm in a microplate reader (Bio-Rad Laboratories, USA).

Statistical analysis

The descriptive analysis (mean and standard deviation) was used to discuss the results, assuming the normal distribution of the studied variables. The t-test was used for the antimicrobial activity test. The value of $p<0.05$ was used to determine a significant difference. All calculations were performed using the Statistical program 8.0.

RESULTS

Phytochemical and antioxidant analysis

The qualitative phytochemical screening of the methanolic extract (CgME) and its fractions by TLC revealed the presence of all tested secondary metabolites classes, except for alkaloids. The hexane fraction (CgHF) concentrated the terpenes and steroids, while the ethyl acetate fraction (CgEF) enriched all secondary metabolite classes, mainly flavonoids, and proanthocyanins. The methanolic fraction (CgMF) presents the same profile of the CgME, except for the absence of proanthocyanins (Table 1).

The quantification of total phenolic compounds and flavonoids in the CgEM and its fractions confirmed the enrichment of the CgEF, which present higher levels of both secondary metabolite classes, as shown in Table 2. The antioxidant activity of the extract and its fractions were also evaluated through two assays: total antioxidant activity (phosphomolibdenium assay) and DPPH free radical scavenging assay. In the first test, CgEF presented the best results, followed by CgME and CgMF. In DPPH free radical scavenging assay, CgME and CgEF showed similar EC_{50} values. In both assays, the CgHF showed only residual activity (Table 2).

Biological activities

Cytotoxicity against cancer cell lines

The extract and its fractions were screened at 50 µg/mL against MCF-7, NCIH292, and HL-60 cancer cell lines. The CgME, CgEF, and CgMF inhibited less than 50% of cell growth, while CgHF inhibited 74.5 ± 3.4% and 90.9 ± 4.0% for NCI-H292 and HL-60, respectively. The IC_{50} for these two cell lines was 19.49 (12.65-30.01) and 0.94 (0.74-1.19) µg/mL for NCI-H292 and HL-60, respectively. Doxorubicin, used as control, showed IC_{50} of 0.1 (0.092 – 0.110) and 0.06 (0.054-0.063) µg/mL for NCIH292 and HL-60, respectively.

Table 1. Phytochemical screening of the methanolic extract from *Cassia grandis* stem bark (CgME) and its hexane (CgHF), ethyl acetate (CgEF), and methanolic (CgMF) fractions.

Secondary metabolite classes	CgME	CgHF	CgEF	CgMF
Mono and sesquiterpenes	-	++	++	-
Triterpenes and sterols	-	++	++	-
Saponins	+	-	+	+
Flavonoids	+++	-	+++	++
Coumarins	+	-	+	+
Phenylpropanoids	+	-	+	+
Anthraquinones	+	-	+	+
Alkaloids	-	-	-	-
Proanthocyanins	+	-	+++	-

(-) negative; (+) weak; (++) medium; and (+++) strong.

Table 2. Total phenolic and flavonoid content and antioxidant activity of the methanolic extract from *Cassia grandis* stem bark (CgME) and its hexane (CgHF), ethyl acetate (CgEF), and methanolic (CgMF) fractions.

Extract	Phenolics (mg GAE/g)	Flavonoids (mg QE/g)	P-Mo (%)	$DPPH^{\bullet}$ (EC_{50})
CgME	274.6 ± 4.4	14.7 ± 2.5	82.7 ± 2.2	4.34(4.09 - 4.60)
CgHF	-	-	23.1 ± 1.9	>50
CgEF	288.4 ± 5.6	37.2 ± 1.2	88.7 ± 1.1	4.29 (4.16 - 4.41)
CgMF	256.2 ± 0.6	9.7 ± 0.9	72.9 ± 1.8	9.10 (8.56 - 9.69)
Gallic acid	-	-	-	< 1.56

Antimicrobial activity

A disk diffusion assay against a panel of micro-organisms was used to screen the antimicrobial activity of the extract and its fractions. The CgME presented inhibition zone diameters of 15.3 ± 0.6 mm for *S. aureus*, *E. coli*, and *M. smegmatis*, and of 13.3 ± 0.6 mm for *B. subtilis*. After the fractionation, the antimicrobial activity against *E. coli* and *M. smegmatis* was lost, while the CgEF showed an increase in the activity against *S. aureus* and *B. subtilis*, with inhibition zone diameters of 17.3 ± 0.6 and 16.3 ± 0.6 mm, respectively. CgHF was not active for any tested micro-organisms. Kanamycin antibiogram disks (30 µg/disk) were used as control, presenting inhibition zone diameters among 13.0 ± 0.5 and 40.0 ± 1.0 mm for the tested micro-organisms.

As the next step, MIC and MBC values of the CgEM and the active fractions (CgEF and CgMF) were determined against *S. aureus* multidrug-resistant clinical isolates. MICs between 250 and 500 µg/mL were obtained for all extract/fractions and tested strains. On the other hand, MBC varied from 250 to 2,000 µg/mL, with the best results obtained for CgEF (Table 3).

Schistosomicidal activity

Table 4 shows the mortality kinetics of adult *S. mansoni* worm couples treated with *C. grandis* methanolic extract and its fractions incubated for 24, 48, and 72 h. During the 72 h incubation time, worm couples treated only with culture medium showed typical body movements, with peristalsis of the internal organs, suckers' movements, and adhering to the culture plate (score 3). CgME killed 18.75% of the worms at 400 µg/mL after 72 h of exposure. The fractionation of the extract enhanced its schistosomicidal activity, with at least 75% mortality for all fractions at 72 h. After 24 and 48 h of incubation, worm couples treated with PZQ were immobile (score 0) showing 87.5 and 100% mortality, respectively.

The cell viability of *S. mansoni* worms after 72 h incubation time was determined by the mitochondrial reduction of MTT. The CgME (400 µg/mL) reduced the cell viability by 33.8%, while the CgEF, the most active fraction, at 400 and 300 µg/mL reduced the cell viability by 97.5 and 85.3%, respectively. PZQ reduced cell viability by 80.7% (Figure 1).

Chemical composition of the active fractions (CgHF and CgEF)

Quantitative chemical analysis of CgHF by GC-MS revealed the presence of 8 ubiquitous compounds (fatty acids, phytosterols, and triterpenes). The major compounds were clionasterol (23.9%), lupeol acetate

Table 3. Antimicrobial activity of the methanolic extract from *Cassia grandis* stem bark (CgME) and its ethyl acetate (CgEF) and methanolic (CgMF) fractions against multidrug-resistant *S. aureus* clinical isolates.

S. aureus	MIC (MBC) µg/mL		
	CgEM	CgEF	CgMF
UFPEDA 01	250 (2000)	250 (250)	250 (2000)
UFPEDA 679	500 (1000)	500 (1000)	500 (2000)
UFPEDA 700	500 (1000)	250 (500)	500 (500)
UFPEDA 719	250 (500)	250 (250)	250 (250)

Minimal inhibitory concentrations (MIC) and minimal bactericide concentration (MBC) are expressed in µg/mL.

Table 4. Motility score of control worms, treated with praziquantel (PQZ – 3.12 µg/ml), and with the methanolic extract from *Cassia grandis* stem bark (CgME) and its hexane (CgHF), ethyl acetate (CgEF), and methanolic (CgMF) fractions (400 - 50 µg/mL) after 24, 48, and 72 h of incubation.

Group	Incubation period (h)														
	24 h					48 h					72 h				
	% of worm uncoupling	Percent of worms in motility scores				% of worm uncoupling	Percent of worms in motility scores				% of worm uncoupling	Percent of worms in motility scores			
		0	1	2	3		0	1	2	3		0	1	2	3
Control															
RPMI[1]	0				100	0				100	0				100
DMSO[2]	0				100	0				100	0				100
PZQ															
3.12	0	87.5	12.5				100								
CgME															
400	25		31.25	68.75		37.5		100			62.5	18.75	81.25		
300	0		12.5	87.5		25		100			50	12.5	87.5		
200	0			25	75	0			50	50	25		25	75	
100	0				100	0			81.25	18.75	0			100	
50	0				100	0				100	0				100
CgHF															
400	37.5		100			100	62.5	37.5			100	100			
300	0			75	25	0		31.25	68.75		50	43.75	56.25		
200	0			100		0			87.5	12.5	25		37.5	62.5	
100	0				100	0				100	0			25	75
50	0				100	0				100	0				100

Table 4. Contd.

CgEF																
400	50	43.75	56.25			75	87.25	12.5			100	100				
300	50		62.5	37.5		50	6.25	93.75			100	81.25	18.75			
200	37.5		31.25	68.75		50		25	75		75	12.25	87.5			
100	0				100	0				100	25		37.5	62.5		
50	0				100	0				100	0				100	
CgMF																
400	50	12.5	37.5	50		100	56.25	43.75			100	75	25			
300	50	12.5	25	62.5		100	50	50			100	62.5	37.5			
200	50		18.75	81.25		100		31.25	68.75		100	37.5	50	12.5		
100	0			100		62.5			62.5	37.5	62.5			81.25	18.75	
50	0				100	50				100	37.5			12.5	87.5	

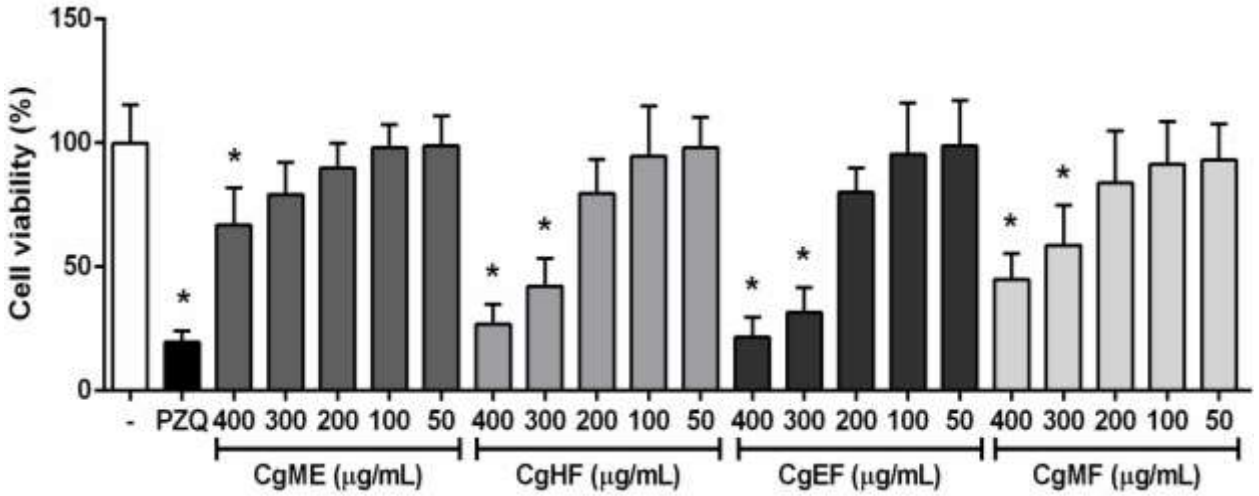

Figure 1. *In vitro* effects of the methanolic extract from *Cassia grandis* stem bark (CgME) and its hexane (CgHF), ethyl acetate (CgEF) and methanolic (CgMF) fractions (400 - 50 µg/mL) and praziquantel (PZQ, 3.12 µg/mL) on cell viability of adult *Schistosoma mansoni* couple worms after 72 h of incubation. The results were expressed as mean ± standard deviation (SD) and analyzed by ANOVA, followed by Tukey's test. *$p < 0.05$ compared to the control (-).

Table 5. Chemical composition of the hexane fraction (CgHF) from the methanolic extract from *Cassia grandis* stem bark.

Peak	Retention time (min)	Compound	Area (%)
1	3.020	o-Xylene	6.54
2	3.099	p-Xylene	3.29
3	15.206	Methyl palmitate	1.61
4	15.493	Palmitic acid	15.17
5	16.416	Linoleic acid	16.16
6	23.142	Campesterol	3.09
7	23.503	Stigmasterol	6.74
8	24.309	Clionasterol	23.94
9	25.617	Lupeol acetate	18.71
10	25.775	β-simiarenol	4.75
Total (%)			**100.00**

Table 6. Chemical composition of the ethyl acetate fraction (CgEF) from the methanolic extract from *Cassia grandis* stem bark.

Peak	Retention time (min)	m/z	Compound
1	2.61	305.07	(epi)gallocatechin
2	3.34	305.07	(epi)gallocatechin
3	3.45	289.07	(epi)catechin
4	4.06	289.07	(epi)catechin
5	5.63	301.07	Quercetin
6	6.50	285.04	Luteolin
7	8.84	384.09	Piperidine derivative

(18.7%), linoleic (16.2%) and palmitic (15.2%) acids (Table 5). One can check the GC-MS chromatogram and mass spectra of all compounds in Figures S1-S11 (Supplementary Material). Qualitative chemical analysis of CgEF by UPLC-MS was able to identify 6 flavonoids (Clarke et al., 2014). A piperidine derivative was also proposed based on its occurrence in *Cassia* sp. (Pivatto et al., 2005) The compounds found in CgEF are shown in Table 6 and in Figure S12-S16 (Supplementary Material).

DISCUSSION

Cassia species play an important role in traditional medicine around the world, with reports of use in Chinese and ayurvedic medicines to treat several diseases, mainly due to their purgative effects (Prada et al, 2014). Many secondary metabolite classes have been found in different *Cassia* species, such as flavonoids, tannins, terpenes and sterols, saponins, alkaloids, and specially anthraquinones (Dave and Ledwani, 2014). The same chemical profile here described for the methanolic extract of the *C. grandis* stem bark was previously reported for *C. grandis* leaves and fruits (Meena, 2010; Prada et al., 2018). A study with the leaves methanolic extracts of seven *Cassia* spp. native from Egypt highlights *C. grandis* as the species with higher content of phenolic compounds (169.73 ± 2.9 mg GAE/g extract) and flavonoids (39.03 ± 1.0 mg QE/g extract). Here, higher values were found for phenolic compounds in the CgME and two of its fractions, CgEF and CgMF. On the other hand, only CgEF showed similar levels of flavonoids.

Oxidative stress is one of the main causes of human diseases and many phytochemicals, such as flavonoids and tannins, have been reported to prevent oxidative damage (Amaral et al., 2019). *In vitro* antioxidant activity of CgME and its fractions was carried out through phosphomolibdium and DPPH assays, which are methods frequently used to determine the radical-scavenging activity of natural products. In this study, CgME and its fraction CgEF showed EC_{50} of approximately 4.3 µg/mL, agreeing with the values published by Prada et al. (2018) for the extracts of *C. grandis* fruits. The study performed by El-Hashash et al. (2010) also reports total antioxidant activity (TAA) for the same seven *Cassia* spp., where *Cassia glauca* presented the highest TAA with 53.4% followed by *C. grandis* with 24% in relation to the activity of ascorbic acid alone. In our study, we reported values greater than 70% for all extract/fractions, except for CgHF which presented only

residual activity of about 23%.

The poor cytotoxicity of CgME and its fractions, CgEF and CgMF, is probably due to the high levels of phenolic compounds and flavonoids, which are cytoprotective. Hegazi and Hashim (2016) reported the low cytotoxicity of the polar fractions of *C. grandis* leaves against three different cancer cell lines, hepatocellular (HepG-2), breast (MCF- 7), and prostate (PC3) by the neutral red uptake assay. The nonpolar fraction, CgHF, instead, showed relevant cytotoxicity against two cancer cell lines, NCI-H292, and HL- 60. The major compounds found in the CgHF were clionasterol (γ-sitosterol) and lupeol acetate, in addition to the fatty acids, linoleic and palmitic acids. Several phytosterols have been reported as cytotoxic to cancer cell lines: γ-sitosterol induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells (Sundarraj et al., 2012), while fucosterol, the main metabolite of clionasterol, exhibits selective antitumor anticancer activity against HeLa cell line by inducing mitochondrial- mediated apoptosis, cell cycle migration inhibition and downregulation of mTOR/ PI3K/Akt signalling pathway (Jiang et al., 2018). Lupeol and its esters have also been reported as cytotoxic against tumor cells through the inhibition of key enzymes, such as topoisomerase II, DNA polymerase β, and farnesyltransferase (Gallo et al., 2009). Lupeol acetate is the active principle of *Himatanthus drasticus* latex, known in Brazil as *janaguba*, which is used in folk medicine as an anti-inflammatory and to treat cancer (Lucetti et al., 2010).

The antimicrobial activity of *Cassia* species against Gram-positive bacteria is well documented and corroborates our findings Chanda et al. (2012) evaluated the antimicrobial activity of the polar extracts from *Cassia auriculata*, *Cassia fistula*, *Cassia siamea*, and *Cassia tora*, which were active mainly against *S. aureus*. Regasini et al. (2010) proposed a CMI-based classification for plant extracts, being considered those with CMI < 100 µg/mL as highly active; between 100-500 µg/mL as moderately active; from 500 to 1000 µg/mL as poorly active; and above 1000 µg/mL as inactive. Based on this classification, CgEM and its polar fractions, CgEF and CgMF, were considered moderately active for both standard and clinical isolate strains. Proestos et al. (2005) studied 27 Greek plants in which they identified several flavonoids, including quercetin, apigenin, luteolin, rutin, catechin, epicatechin, and naringenin. These compounds presented antimicrobial activity against *E. coli*, *S. aureus*, *Salmonella enteritidis*, *Listeria monocytogenes*, and *Bacillus cereus*. The chemical analysis of CgEF, which was the most active fraction, revealed the presence of gallocatechin/epigallocatechin, catechin/epicatechin, quercetin, and luteolin, corroborating the previously published results (Proestos et al., 2005).

Despite being used in folk medicine against worms and intestinal parasites, there is no report in the literature of the anti-*S. mansoni* activity of any *Cassia* species. Here, it was possible to correlate the enrichment in phenolic compounds and flavonoid levels with the increase in schistosomicidal activity of the CgEF when compared to the CgME. Many flavonoids and phenolic acids, including kaempferol, gallic acid, and protocatechuic acid, have been reported in literature for their ability to impair reproduction and even kill adult *S. mansoni* worms, causing tegument damage and reducing egg production (Braguine et al., 2009). The anthelmintic effect of the flavonoid luteolin, isolated from *Ajania nubigena* (Wall.) C.Shih (Asteraceae) and also present in the CgEF, against *S. mansoni* and *Trichuris muris* has been reported by Wangchuk et al. (2016). In addition to killing adult worms, luteolin was effective against schistosomula (IC_{50} 13.3 µg/mL), the juvenile stage of *S. mansoni* which is refractory to praziquantel.

Another class of secondary metabolites with strong and diverse biological properties are the alkaloids, which can cross biological membranes and accumulate in specific sites due to deprotonation/protonation of their nitrogen atoms. Piperidine alkaloids are very common in *Cassia* and *Senna* species, and for that reason we proposed piperidine derivative structure to the peak [M-H]- 384.09. Castro et al. (2016) demonstrated the *in vitro* schistosomicidal activity of (−)-cassine/(−)-spectaline, the two major alkaloids found in *Senna spectabilis* flowers, against adult worms and cercariae.

Conclusion

The methanolic extract of *C. grandis* stem bark and its fractions showed *in vitro* antioxidant activity due to their high phenolic and flavonoid contents. The non-polar fraction CgHF was cytotoxic to two cancer cell lines and this effect was correlated to the presence of clionasterol and lupeol acetate, a phytosterol and a triterpene ester known for their antitumor effects. The ethyl acetate fraction (CgEF) exhibited both antimicrobial and schistosomicidal activities against multidrug-resistant *S. aureus* and *S. mansoni*, respectively, which have been attributed to the presence of flavonoids, such as catechin derivatives, quercetin, and luteolin in the CgEF. These results agree with the popular uses of *C. grandis* and should stimulate future research on this species.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATIONS

CgME, *Cassia grandis* methanolic extract; **CgHF,** *Cassia grandis* hexane fraction; **CgEF,** *Cassia grandis* ethyl acetate fraction; **CgMF,** *Cassia grandis* methanolic fraction; **CFU,** colony-forming unit; **DPPH,** 2,2-Diphenyl-1-picrylhydrazyl; **GAE,** gallic acid equivalent; **GC-MS,** Gas chromatography-mass spectrometry; **HL-60,** human promyelocytic leukemia cell line; **MCF-7,** human breast adenocarcinoma cell line; **MBC,** minimum bactericidal concentration; **MIC,** minimum inhibitory concentration; **MTT,** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **NCI-H292,** human lung mucoepidermoid carcinoma cell line; **PZQ,** praziquantel; **QE,** quercetin equivalent; **TLC,** Thin-layer chromatography; **UPLC-MS,** Ultra-performance liquid chromatography-mass spectrometry.

REFERENCES

Andrade ALP, Miotto STS, Santos EPA (2009). Subfamília Faboideae (Fabaceae Lindl.) no Parque Estadual do Guartelá Paraná, Brasil. Hoehnea 36(4):737-768.

Adams RP (2001). Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured Publishing Corporation, 3rd Ed, Carol Stream.

Aires AL, Ximenes ECPA, Silva RAR, Barbosa VX, Góes AJS, Peixoto CA, Souza VMO, Albuquerque MCPA (2014). Ultrastructural Analysis of β-lapachone-induce surface membrane damage in male adult *Schistosoma mansoni* BH strain worms. Experimental Parasitology 142:83-90.

Amaral GP, Mizdal CR, Stefanello ST, Mendez ASL, Puntel RL, Campos MMA, Soares FAA, Fachinetto R (2019). Antibacterial and antioxidant effects of *Rosmarinus officinalis* L. extract and its fractions. Journal of Traditional Complementary Medicine 9(4):383-392.

Bauer AW, Kirby WMM, Shevis JC, Turck M (1966). Antibiotic susceptibily testing by a standardized single disc method. American Journal of Clinical Pathology 45(4):493-496.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature 181:1199-1200.

Braguine CG, Costa ES, Magalhães LG, Rodrigues V, da Silva Filho AA, Bastos JK, Silva ML, Cunha WR, Januário AH, Pauletti PM (2009). Schistosomicidal evaluation of Zanthoxylum naranjillo and its isolated compounds against Schistosoma mansoni adult worms. Nature A Journal Biosciences 64(11-12):F793-F797.

Calixto JB (2019). The role of natural products in modern drug discovery. Anais da Academia Brasileira de Ciências 91(3).

Castro AT, Castro AP, Silva MS, Souza IMM, Martins-Souza RL, Chagas-Paula DA, Coelho LFL, Bolzani VS, Pivatto M, Junior CV, Marques MJ (2016). In vitro evaluation of the schistosomicidal effect of the extracts, fractions and major 3-hydroxy-2,6-dialkyl-substituted piperidine alkaloids from the flowers of *Senna spectabilis* (Fabaceae). Bioorganic and Medicinal Chemistry Letters 26(17):4197-4204.

Chanda S, Kaneria M, Baravalia Y (2012). Antioxidant and antimicrobial properties of various polar solvent extracts of stem and leaves of four *Cassia* species. African Journal Biotechnology 11(10):2490-2503.

Clarke KA, Dew TP, Watson REB, Farrar MD, Bennett S, Nicolaou A, Rhodes LE, Williamson G (2014). High performance liquid chromatography tandem mass spectrometry dual extraction method for identification of green tea catechin metabolites excreted in human urine. Journal Chromatography B 972:29-37.

Clinical and Laboratory Standards Institute (CLSI) (2018). M100-S29 performance standards for antimicrobial susceptibility testing 29th informational supplement. Available at: https://clsi.org/media/2663/m100ed29_sample.pdf

Dave H, Ledwani L (2012). A review on anthraquinones isolated from *Cassia* species and their applications. Indian Journal of Natural Products and Resources 3(3):291-319.

El-Hashash MM, Abdel-Gawad MM, El-Sayed MM (2010). Antioxidant properties of methanolic extracts of the leaves of seven Egyptian *Cassia* species. Acta Pharmaceutica 60(3):361-367.

Forzza RC, Leitman PM, Costa AF, Carvalho jr. AA, Peixoto A, Walter BMT, Bicudo C, Zappi D, Costa DP, Lleras E, Martinelli G, Lima HC, Prado J, Stehmann JR, Baumgratz JFA, Pirani JR, Sylvestre L, Maia LC, Lohmann LG, Queiroz LP, Silveira M, Coelho MN, Mamede MC, Bastos MNC, Morim MP, Barbosa MR, Menezes M, Hopkins M, Secco R, Cavalcanti TB, Souza VC (2010). Introdução. In: Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available at: http://floradobrasil.jbrj.gov.br/2010/

Gallo MBC, Miranda B, Sarachine MJ (2009). Biological Activies of Lupeol. International Journal of Research in Pharmaceutical and Biomedical Sciences 3(1):46-66.

Guidoti DGG, Guidoti DT, Romero AL, Ruiz ALTG, Foglio MA, Carvalho JE, Rocha CLMSC (2019). Kaurenoic acid from Annona squamosa L. exhibits antiproliferative effect on human tumor cell lines and induces apoptosis in *Aspergillus nidulans*. Revista Fitos 13(2):122-136.

Hegazi NM, Hashim AN (2016). Grandisin, 2-methoxy 6,7,2',6'-tetrahydroxy flavanone 6-O-glucoside, from *Cassia grandis* leaves - antioxidant and cytotoxic activities. Pharmazie 71(9):544-547.

Horiuchi A, Satou T, Akao N, Koik K (2005). The effect of free and polyethylene glycol-liposome-entrapped albendazole on larval mobility and number in *Toxocara canis* infected mice. Veterinary Parasitology 129(1-2):83-87.

Jiang H, Li J, Chen A, .Li Y, Xia M, Guo P, Yao S, Chen S (2018). Fucosterol exhibits selective antitumor anticancer activity against HeLa human cervical cell line by inducing mitochondrial mediated apoptosis, cell cycle migration inhibition and downregulation of m-TOR/PI3K/Akt signalling pathway. Oncology Letters 15(3):3458-3463.

Joshi H, Kapoor VP (2003). *Cassia grandis* Linn. F. Seed galactomannan: structural and crystallographical studies. Carbohydrate Research 338(18):1907-1912.

Lima HC, de Queiroz LP, Morim MP, Souza VC, Dutra VF, Bortoluzzi RLC, Iganci JRV, Fortunato RH, Vaz AMSF, Souza ER, de Filardi FLR, Valls JFM, Garcia FCP, Fernandes JM, Martins-da-Silva RCV, Perez APF, Mansano VF, Miotto STS, Tozzi AMGA, Meireles JE, Lima LCP, Oliveira MLAA, Flores AS, Torke BM, Pinto RB, Lewis GP, Barros MJF, Schütz R, Pennington T, Klitgaard BB, Rando JG, Scalon VR, Cardoso DBOS, Costa LC, da,Silva MJ, da,Moura TM, Barros LAV, de,Silva MCR, Queiroz RT, Sartori ALB, Camargo R.A, Lima IB, Costa J, Soares MVB, Snak C, São-Mateus W, Falcão M, J.,Martins MV, Reis IP, Cordula E (2015). Fabaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia 66(4):1085-1113.

Lodha SR, Joshi SV, Vyas BA, Bhavin AV, Mohini CU, Megha SK, Shweta SS, Sheetal KK, Manasi VR (2010). Assessment of the antidiabetic potential of *Cassia grandis* using an in vivo Model. Journal of Advanced Pharmaceutical Technololy Research 1(3):330-333.

Lucetti DL, Lucetti ECP, Bandeira MAM, Helenicy NHV, Aline H Silva, Luzia Kalyne AML, Amanda AL, Victor CCA, Gabriela SS, Gerly AB, Glauce BV (2010). Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated from *Himatanthus drasticus* (Mart.) Plumel. Journal of Inflammation 7:60.

McDonald S, Prenzler PD, Antolovich M, Robards K (2001). Phenolic content and antioxidant activity of olive extracts. Food Chemistry 73:73-84.

Meena M, Jain AK, Jain CP, Gaur K, Kori ML, Kakde A, Nema RK (2009). Screening of Anti-inflammatory and Analgesic Activity of *Cassia grandis* Linn. Acadademic Journal Plant Sciences 2(1):51-55.

Meena MK, Pal SC, Jain AK, Jain CP, Ashawat MS, Gaur K, Jain A (2010). Comparative study on physicochemical variation for different samples of *Cassia grandis* Linn. Leaves. African Journal Plant Science 4(7):261-267.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Aplication to proliferation and cytotoxicity assays. Journal of Immunological Methods 65(1-2):55-63.

Parra AL, Sardiñas MIG (2000). Toxicidadade aguda oral de 3 formas

farmacéuticas a partir de *Cassia grandis* L.. Revista Cubana Planat Medicinales 5(2):68-70.
Pivatto M, Crotti AEM, Lopes NP, Castro-Gamboa I, Rezende A, Viegas Jr. C, Young MCM, Furlan M, Bolzani VS (2005). Electrospray Ionization Mass Spectrometry Screening of Piperidine Alkaloids from *Senna spectabilis* (Fabaceae) Extracts: Fast Identification of New Constituents and Co metabolites. Journal of the Brazilian Chemical Society 16(6B):1431-1438.
Prada AL, Amado JRR, Arranz JCE, Laurido FC (2014). State of the art in *Cassia grandis* L.f. (Cañandonga). Revista Cubana Planat Medicinales 19(1):21-28.
Prada AL, Amado JRR, Keita H, Zapata EP, Carvalho H, Lima ES, De Sousa TP, Carvalho JCT (2018). *Cassia grandis* fruit extract reduces the blood glucose level in alloxan-induce diabetic rats. Biomedicine and Pharmacotherapy 103:421-428.
Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenium complex: specific application to the determination of vitamin E. Analytical Biochemistry 269(2):337-341.
Proestos C, Chorianopoulos N, Nychas GJE, Komaitis M (2005). RP-CLAE Analysis of the phenolics compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. Journal of Agricultural and Food Chemistry 53(4):1190-1195.
Regasini LO, Pivatto M, Scorzoni L, Benaducci T, Fusco-Almeida AM, Giannini MJ, Barreiro EJ, Siva DH, Bolzani VD (2010). Antimicrobial activity of *Pterogyne nitens* Tul., Fabaceae, against opportunistic fungi. Brazilian Journal Pharmacognosy 20(5):706-711.
Singh M, Pandey N, Agnihotri V, Singh KK, Pandey A (2017). Antioxidant, antimicrobial activity and bioactive compounds of *Bergenia ciliata* Sternb.: A valuable medicinal herb of Sikkim Himalaya. Journal of Traditional Complementary Medicine 7(2):152-157.
Stein S, Mallard G, Levitsky A (1997). NIST MS Library Database**.** National Institute of Standards and Technology. Available at: https://www.nist.gov/system/files/documents/srd/NIST1a11Ver2-0Man.pdf
Sundarraj S, Thangama R, sreevani V, Kaveri K, Gunasekaran P, Achiraman S, Kannan S (2012). γ-Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. Journal of Ethnopharmacology 141(3):803-809.
Viegas Junior C, Rezende A, Silva DHS, Castro-Gamboa I, Bolzani VS (2006). Ethnopharmacological, biological and chemical aspects of the *Cassia* genus. Química Nova 29(6):1279-1286.
Woisky RG, Salatino A (1998). Analysis of propolis: some parameters and procedures for chemical quality control. Journal of Apicultural Research 37(2):99-105.
Wangchuk P, Pearson MS, Giacomin PR, Becker L, Sotillo J, Pickering D, Smout MJ, Loukas A (2016). Compounds Derived from the Bhutanese Daisy, *Ajania nubigena*, Demonstrate Dual Anthelmintic Activity against *Schistosoma mansoni* and *Trichuris muris.* PLoS Neglected Tropical Diseases 10 (8).

SUPPLEMENTARY MATERIAL

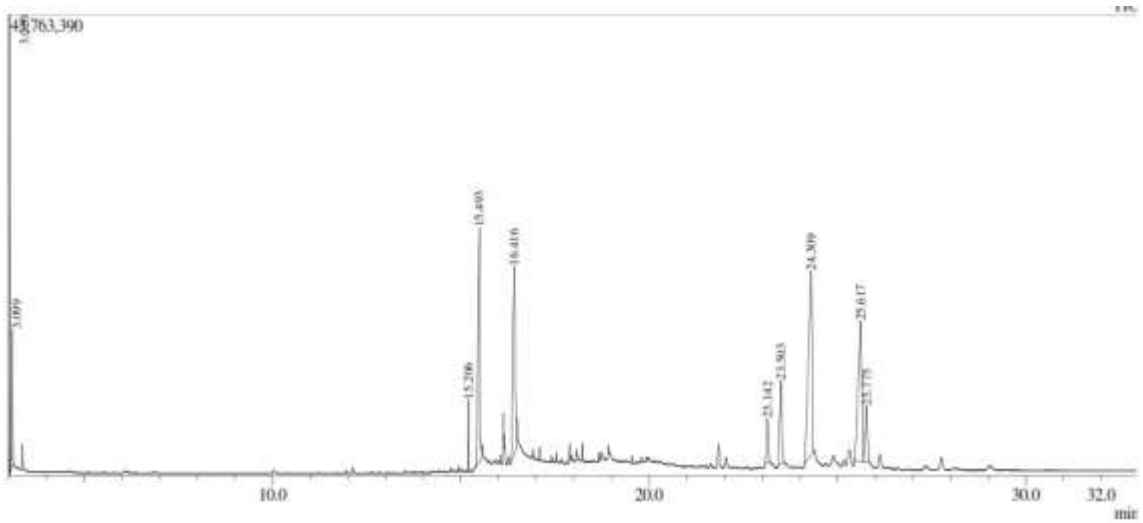
Antibacterial, cytotoxic, and schistosomicidal activities of the methanolic extract from *Cassia grandis* L.f. (Fabaceae) stem bark and its fractions

Table S1. Thin-layer chromatography conditions, standard compounds, and chemical developers used for the phytochemical screening of the methanolic extract from *Cassia grandis* stem bark and its fractions.

Secondary metabolites	Standard compounds	Solvente systems	Chemical developers
Flavonoids and phenylpropanoids	Quercetin, rutin, and chlorogenic acid	EtOAc-HCOOH-AcOH-H_2O (100:11:11:27 v/v)	Neu
Triterpenes and sterols	β-sitosterol	Toluene-EtOAc (90:10 v/v)	Liebermann-Burchard
Mono and sesquiterpenes	Thymol	Toluene-EtOAc (97:3 v/v)	Anisaldehyde Sulfuric Acid
Coumarins and quinones	Coumarin and lapachol	$CHCl_3$-MeOH (98:2 v/v)	KOH
Alkaloids	Pilocarpine	EtOAc -HCOOH-AcOH-H_2O (100:11:11:27 v/v)	Dragendorff and Mayer
Proanthocyanidins	Catechin	EtOAc-HCOOH- AcOH-H_2O (100:11:11:27 v/v)	Vanillin-HCl

Table S2. Multidrug-resistant clinical isolates and their resistance profile.

Clinical isolate	Infection site	Resistance profile
Staphylococcus aureus (UFPEDA 679)	Surgical wound secretion	Erythromycin, clindamycin (Test D positive)
Staphylococcus aureus (UFPEDA 700)	Ulcer secretion	Erythromycin, clindamycin (Test D positive)
Staphylococcus aureus (UFPEDA 719)	Surgical wound secretion	Erythromycin, clindamycin (Test D positive) Ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin, chloramphenicol, tetracycline, penicillin



GCMS Peak Report TIC

Peak#	R.Time	I.Time	Area	Height	A/H	Mark	Name	Base m/z	Area%ght%
1	3.020	3.010	30283834	43636681	0.69		o-Xylene	91.05	6.54 8.75
2	3.099	3.045	15218912	12942409	1.18	V	Benzene, 1,3-dimethyl-	91.00	3.29 8.53
3	15.206	15.175	7437807	6838018	1.09		Hexadecanoic acid, methyl ester	74.00	1.61 4.51
4	15.493	15.395	70228134	22209779	3.16		n-Hexadecanoic acid	73.00	15.17 4.63
5	16.416	16.325	74797174	17876429	4.18		9,12-Octadecadienoic acid (Z,Z)-	67.05	16.16 1.78
6	23.142	23.080	14314930	4198094	3.41		Ergost-5-en-3-ol, (3.beta.)-	43.10	3.09 2.77
7	23.503	23.415	31216435	7925912	3.94		Stigmasterol	55.05	6.74 5.22
8	24.309	24.125	110848305	17489513	6.34		.gamma.-Sitosterol	43.10	23.94 1.52
9	25.617	25.455	86627558	13287338	6.52		Lup-20(29)-en-3-ol, acetate, (3.beta.)-	95.05	18.71 8.75
10	25.775	25.690	21976150	5378623	4.09	V	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.b	259.05	4.75 3.54
			462949239	151782796					100.00 0.00

Figure S1. GC-MS chromatogram and table of constituents from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

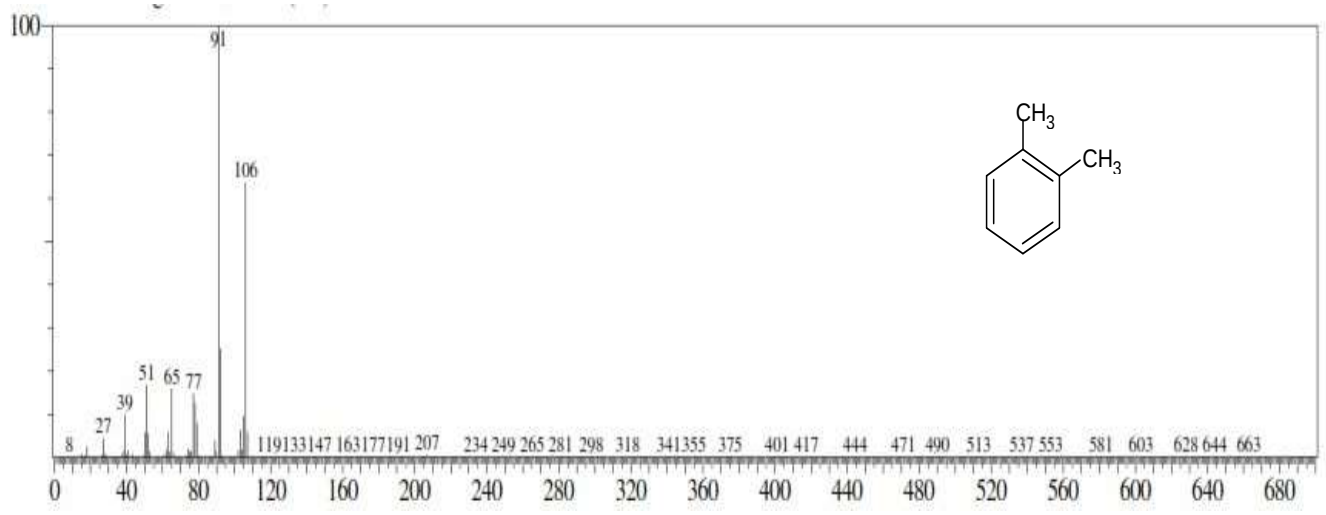


Figure S2. Mass spectrum of compound **1** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

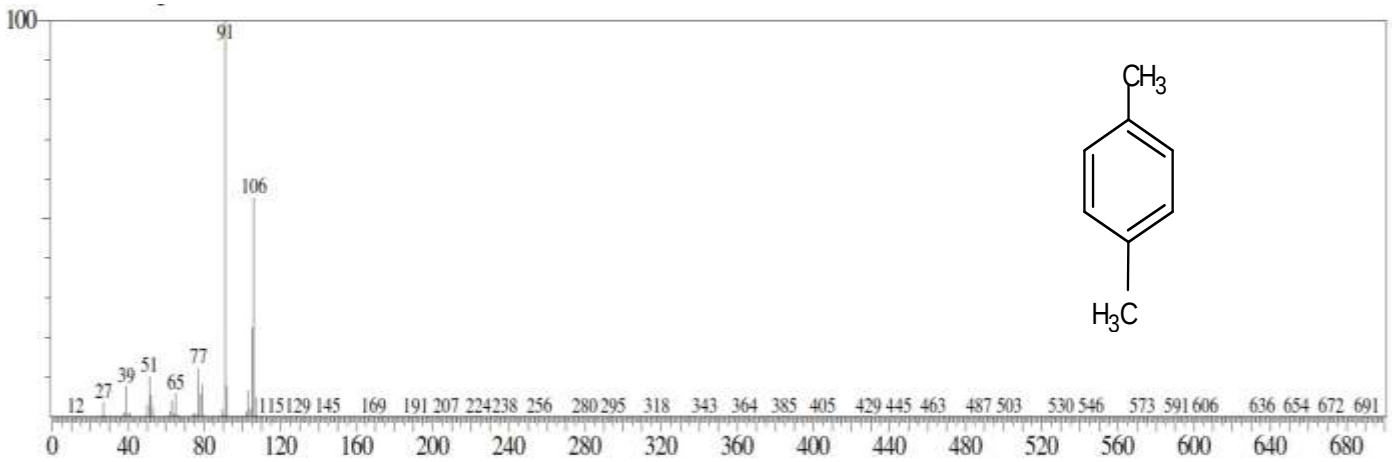


Figure S3. Mass spectrum of compound **2** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

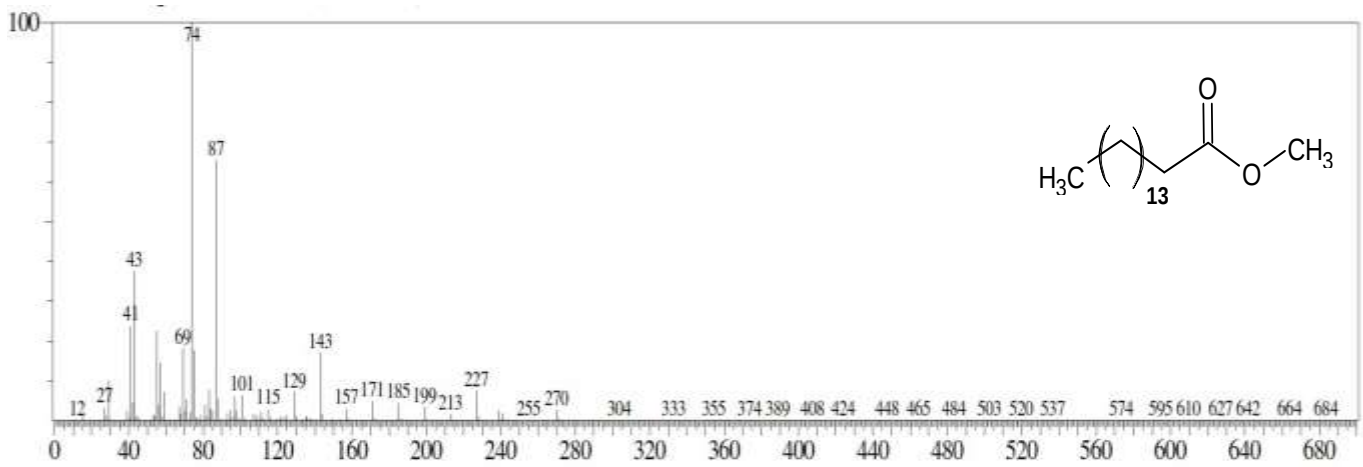


Figure S4. Mass spectrum of compound **3** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

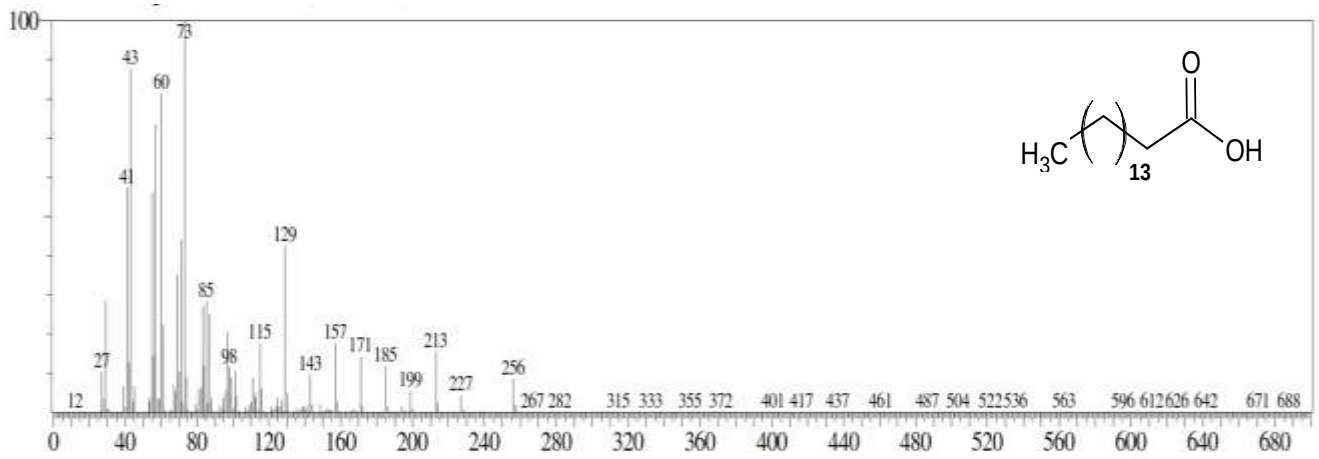


Figure S5. Mass spectrum of compound **4** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

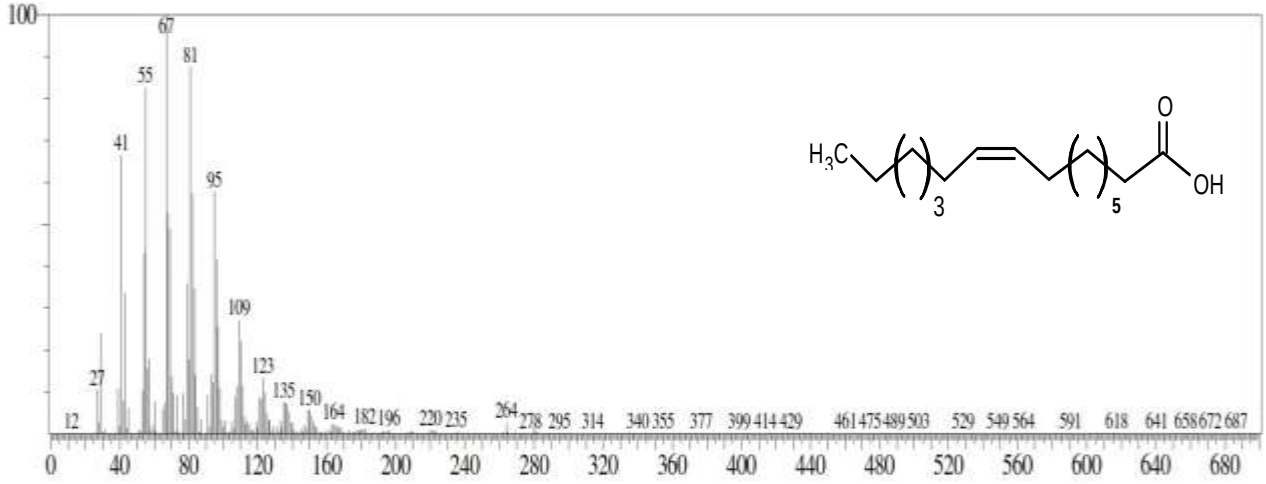


Figure S6. Mass spectrum of compound **5** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

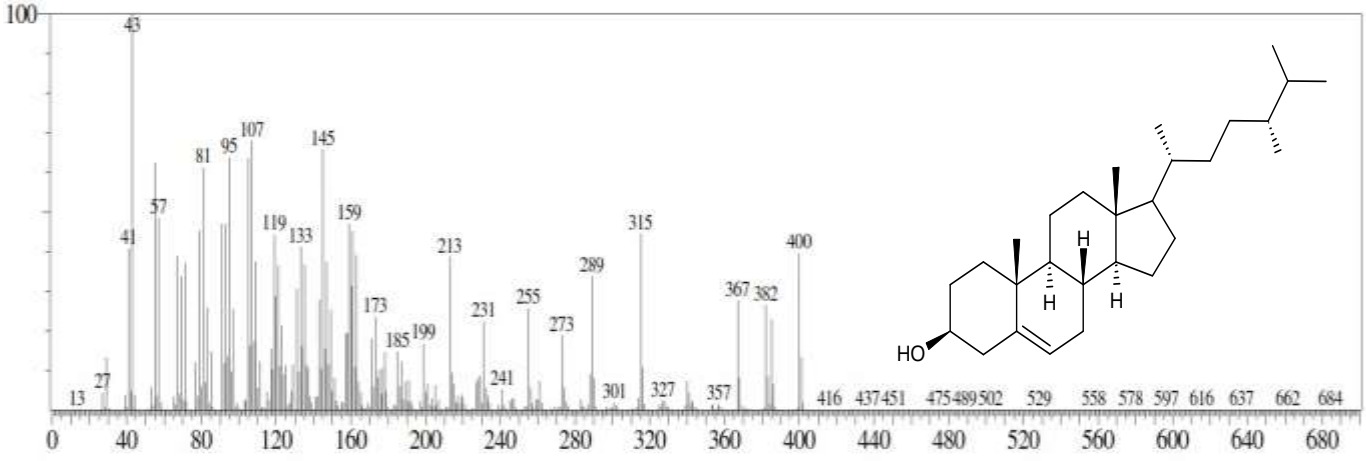


Figure S7. Mass spectrum of compound **6** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

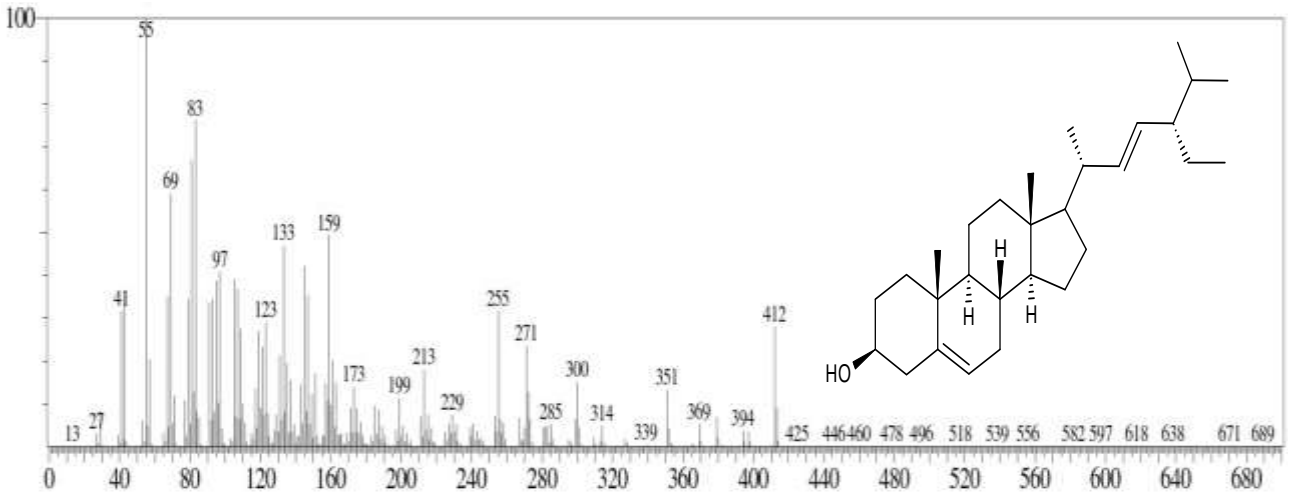


Figure S8. Mass spectrum of compound **7** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

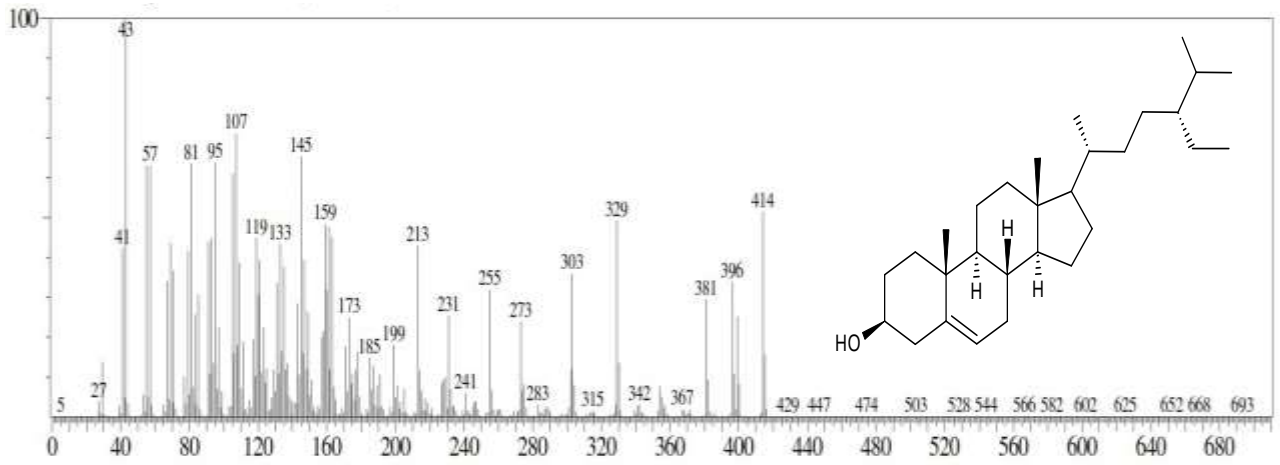


Figure S9. Mass spectrum of compound **8** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

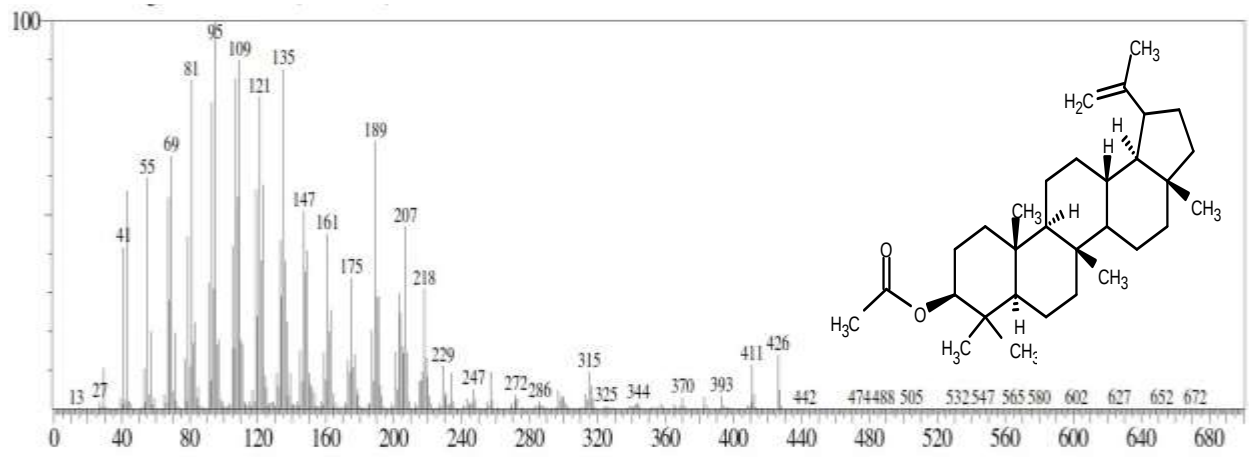


Figure S10. Mass spectrum of compound **9** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

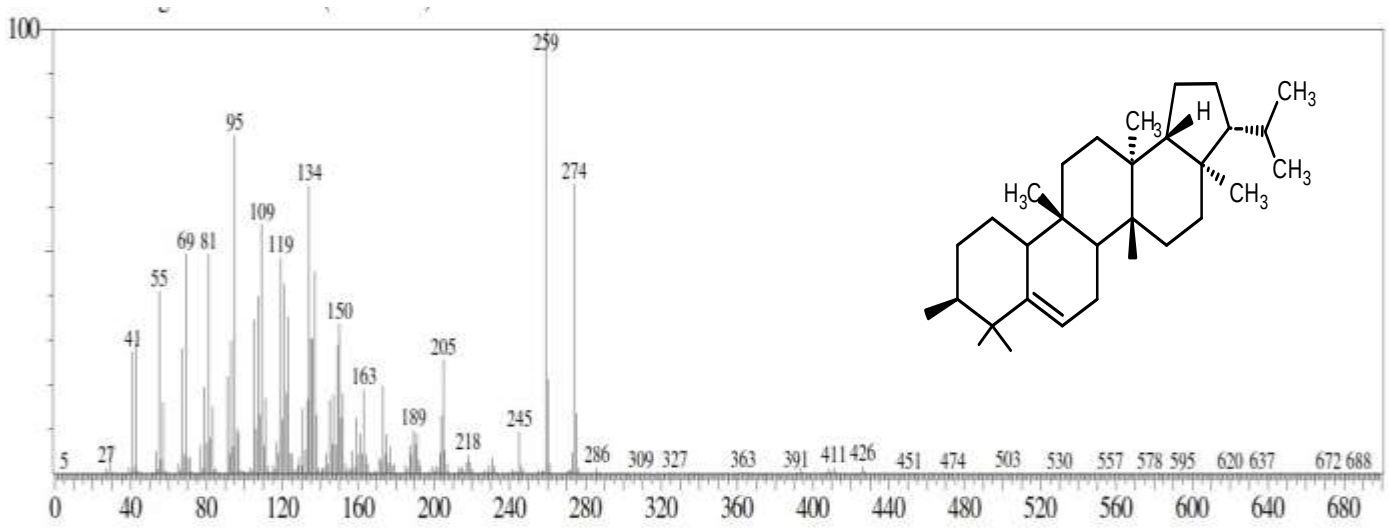


Figure S11. Mass spectrum of compound **10** from the hexane fraction from *Cassia grandis* stem bark methanolic extract.

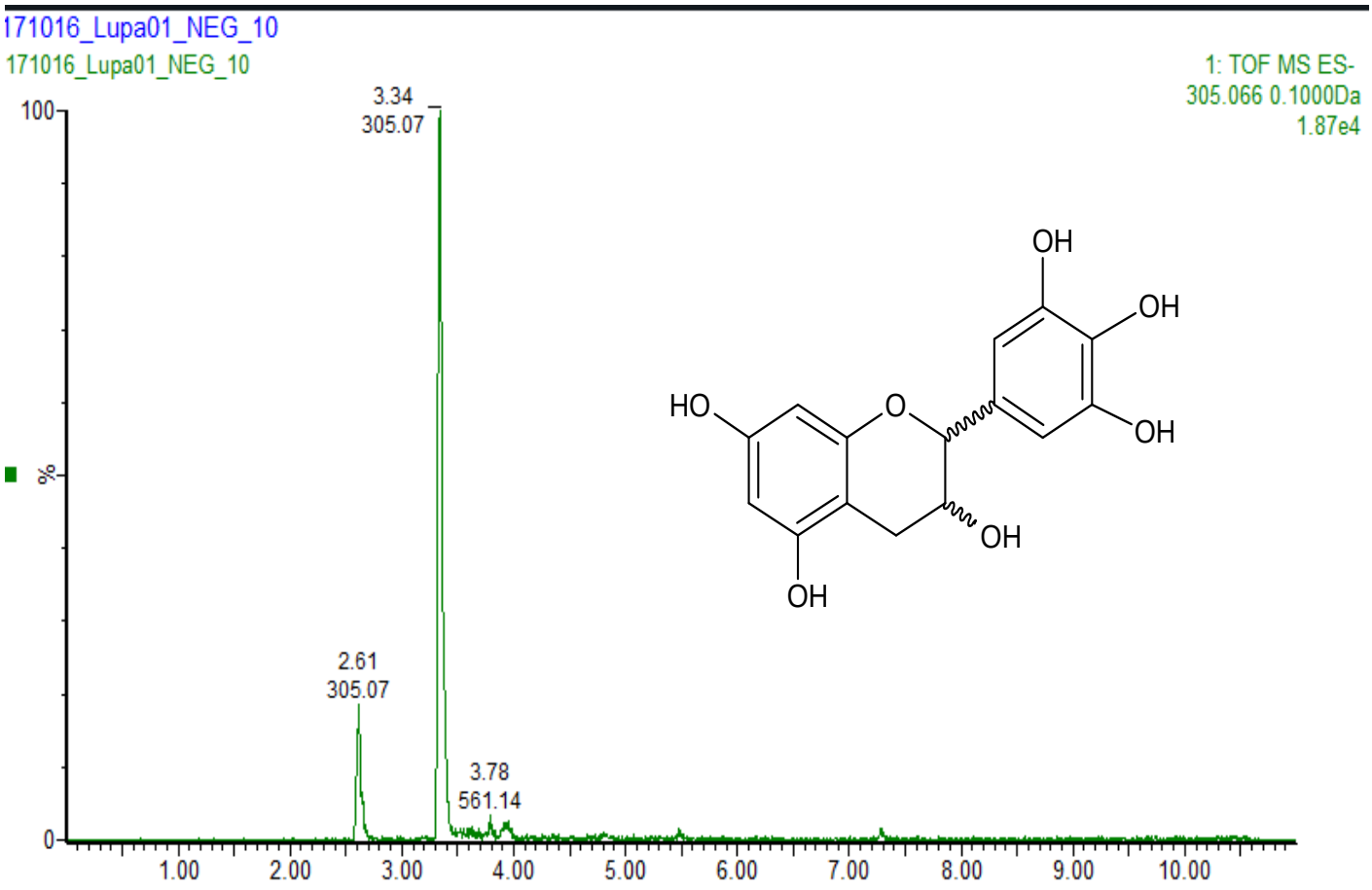


Figure S12. Mass spectrum of Epigallocatechin/Gallocatechin (**1-2**) from ethyl acetate fraction (CgEF) of the methanolic extract from *Cassia grandis* stem bark.

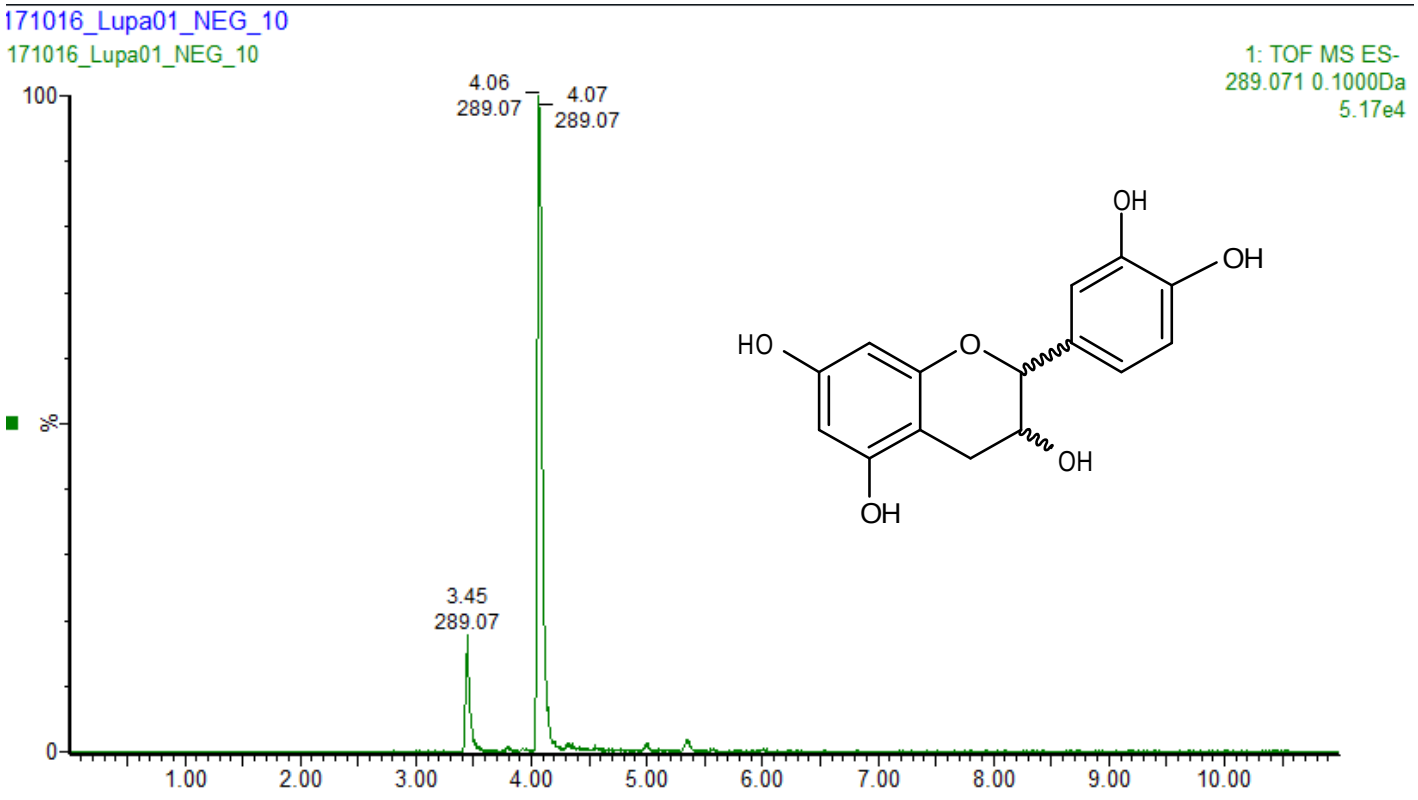


Figure S13. Mass spectrum of Epicatechin/Catechin (**3-4**) from ethyl acetate fraction (CgEF) of the methanolic extract from *Cassia grandis* stem bark.

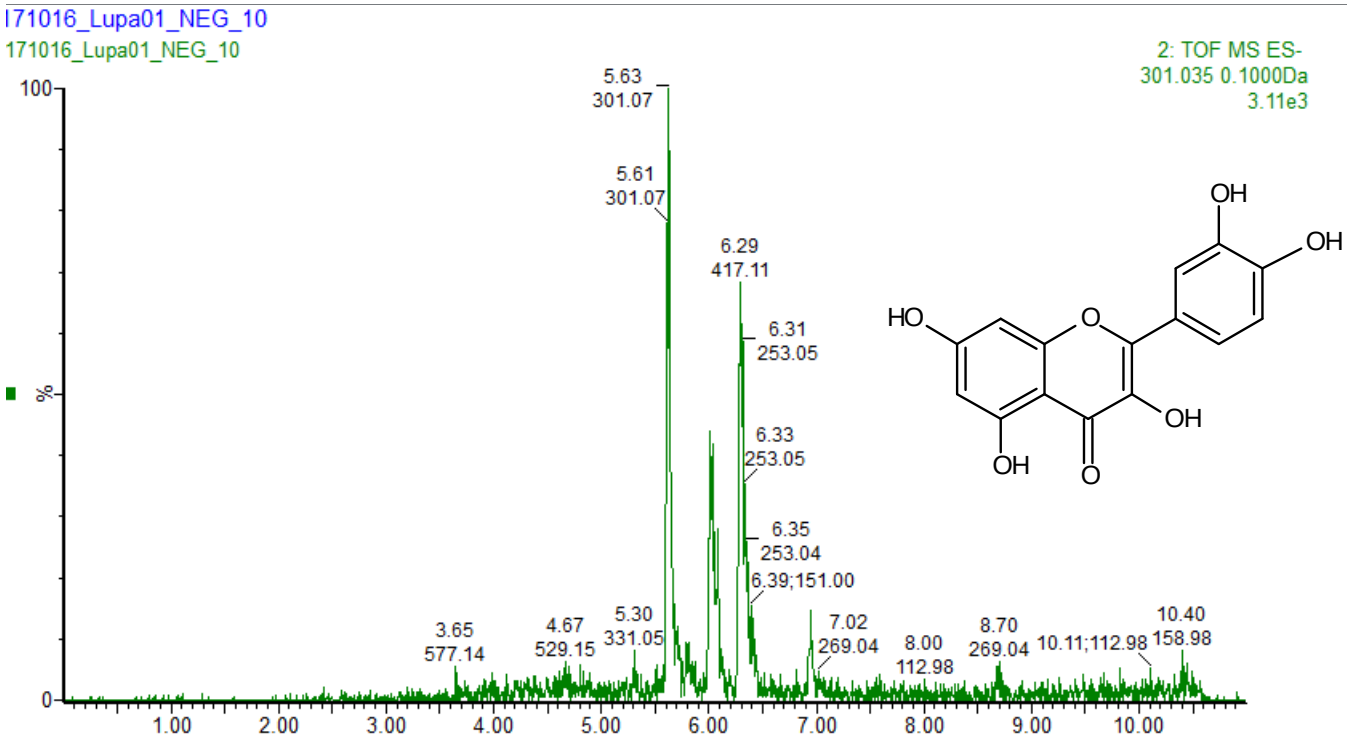


Figure S14. Mass spectrum of Quercetin (**5**) from ethyl acetate fraction (CgEF) of the methanolic extract from *Cassia grandis* stem bark.

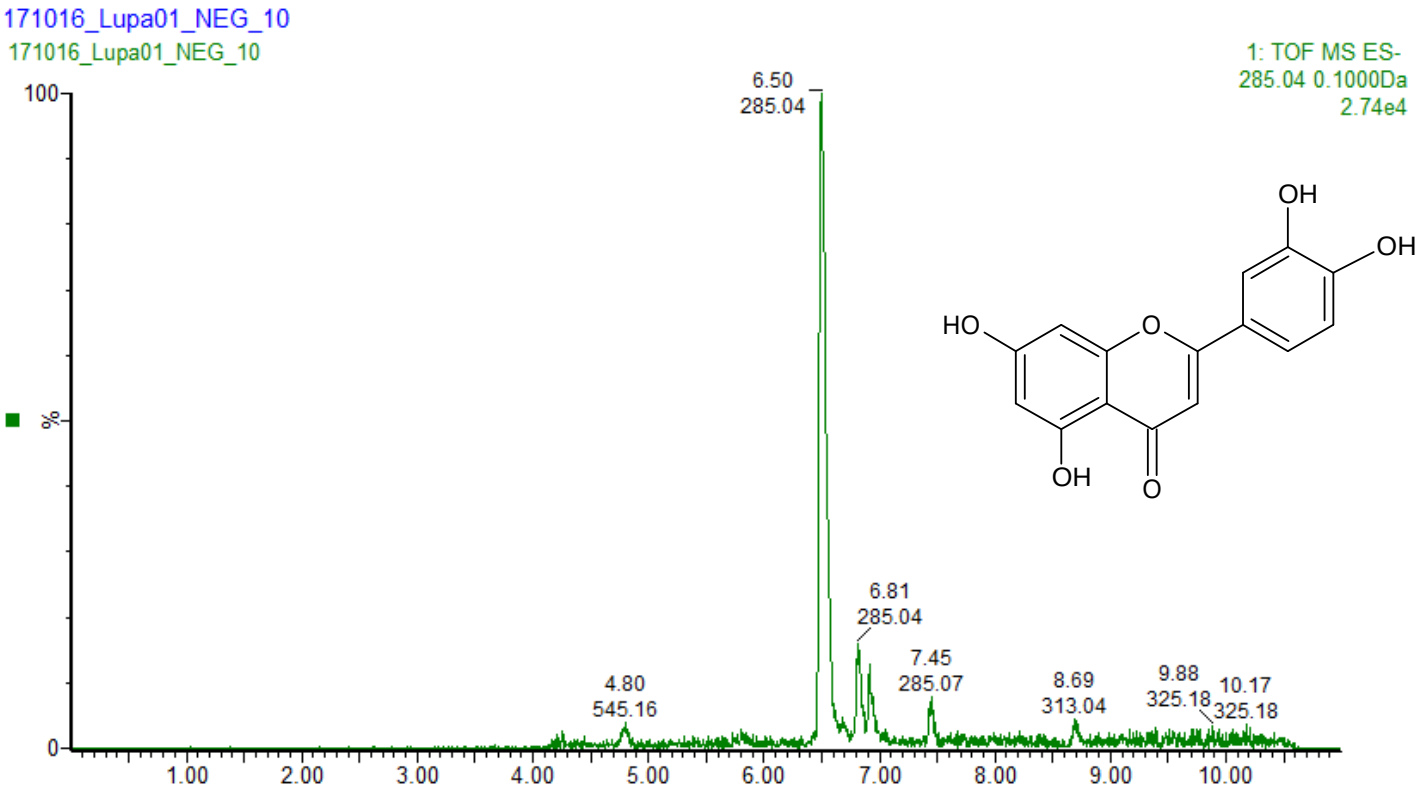


Figure S15. Mass spectrum of Luteolin (**6**) from ethyl acetate fraction (CgEF) of the methanolic extract from *Cassia grandis* stem bark.

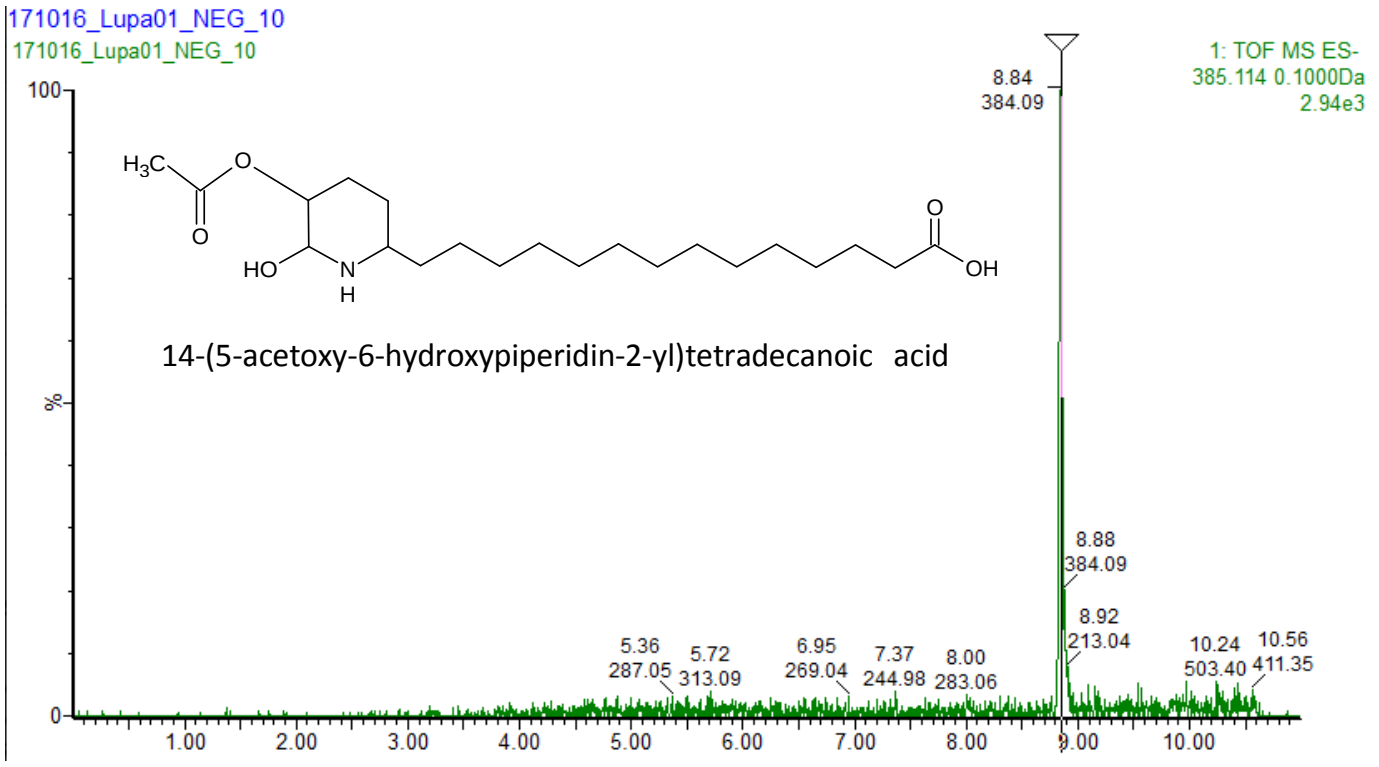


Figure S16. Mass spectrum of piperidine derative (**7**) from ethyl acetate fraction (CgEF) of the methanolic extract from *Cassia grandis* stem bark.