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Chemical constituents and antimicrobial activity of the leaves of *Caryota mitis* Lour. (Arecaceae)

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Abstract
Phytochemical analysis and chromatographic fractionation of the total ethanolic extract of *Caryota mitis* Lour. resulted in isolation and identification of ten compounds, two of them are firstly reported in the family Arecaceae; kaempferol-3-O-rutinoside (8) and chlorogenic acid methyl ester (10), six compounds are firstly reported in the plant; β-amyrin (1), β-sitosterol (2), β-sitosterol-3-O-β-D glucoside (3), kaempferol (4), quercetin (5), kaempferol-3-O-β-D-glucopyranoside (6) and two compounds previously isolated from the plant; quercetin-3-O-β-D-glucopyranoside (7), quercetin-3-O-rutinoside (9). The structures were identified and confirmed through different spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR, EI-MS and UV spectroscopy, in addition to comparison with authentic samples. Antimicrobial assay of the different extracts and fractions revealed strong antibacterial activities on *Staph. aureus* more than *E. coli*; in addition to moderate antifungal activity of n-butanol and aqueous fractions against *Candida albicans*.

Keywords: *Caryota mitis*, Antimicrobial, Arecaceae, flavonoids

Introduction
Medicinal plants have played an essential role in the development of human culture and were directly used as medicines by the majority of cultures all over the world. They are also considered as valuable resources of new drugs due to the adverse side effects of synthetic ones [1]. Large number of plants and their extracts are screened for their characteristic features, chemical constituents and biological activities, among these plants, *Caryota mitis* Lour., which is a member of family Arecaceae.

The family Arecaceae includes about 181 genera with around 2600 species restricted to tropical and subtropical regions [2]. General features of genus *Caryota* include that the leaves are bipinnate, very large and the primary axis terminates in a pair of leaflets but the individual 2^{ry} axis is terminating with an undivided leaflet [3]. Leaflets are more or less triangular with fish tail appearance and usually with several prominent divergent minor ribs instead of single midrib [3]. The fruits of *Caryota mitis* Lour. are non-edible and highly irritant when come in contact with skin due to the presence of acicular crystals of calcium oxalates [4]. Phytochemical screening of the genus *Caryota* revealed that triterpenoids, steroids, flavonoids, alkaloids, saponins, tannins and fatty acids are the chemical constituents present in the plant [5]. In Folk medicine, it was reported that *Caryota mitis* is used to stop vomiting and stomach ache [6], in addition to treatment of constipation, hemorrhoids, loss of virility and rheumatoid arthritis [7]. The present study demonstrates the isolation and identification of the main bioactive constituents of the plant, as well as evaluation of the antimicrobial activity of the different leaves extracts.

Material and Methods
Equipments
Melting points are carried out on an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). Bruker 400 MHz FT NMR spectrometer (Germany), for measuring ^{1}H-NMR, ^{13}C-NMR using DMSO-d_6 and $CDCl_3$ solvents. Column chromatographic separation was performed on silica gel 60 (0.04–0.063 mm, Merck). TLC was performed on precoated TLC plates silica gel 60 F254 (0.2 mm, Merck). The solvent systems used for TLC analysis include n-Hex.: EtOAc (90:10 v/v, S1), CH_2Cl_2: MeOH (90:10 v/v, S2), CH_2Cl_2: EtOAc: MeOH: H_2O (27: 100: 27: 7 v/v, S3) and EtOAc: formic acid: acetic acid: H_2O (100: 11: 11: 26 v/v, S4).

The plant material

Fresh samples of *C. mitis* leaves were collected during the flowering stage in June 2013 from El-Orman Botanical Garden, Giza, Egypt. The plants were kindly identified by Mrs. Traes Labib, general manager of plant taxonomy in El-Orman Botanical Garden, Giza, Egypt.

Extraction and isolation

Five kilograms of the air dried powdered leaves were extracted with 70% ethanol (5×20 L) by maceration at room temperature till exhaustion. The ethanolic extract was concentrated under reduced pressure to give a dark green residue of about 465 g. This alcoholic extract was suspended in distilled water (500 ml) and successively partitioned with n-hexane (10×0.5 L), dichloromethane (5×0.5 L), ethyl acetate (8×0.5 L), n-butanol (5×0.5 L), leaving the aqueous fraction. Each phase was concentrated under reduced pressure to give the corresponding soluble fractions n-hexane fr. (195 g), dichloromethane fr. (3.5 g), ethyl acetate fr. (32 g), n-butanol (15 g) and aqueous fraction (130 g).

About 50 g of the n-hexane fraction was slurred with small amount of silica gel, dried, powdered and transferred to a Buchner (10× 14 cm), eluted using gradient elution system of n-hexane and ethyl acetate, where 5 group fractions (H-I to H-V) were obtained. Group II (9 g) eluted with n-hexane: EtOAc (90:10) was chromatographed on silica gel column using Hex.: EtOAc in gradient elution, followed by crystallization to afford two pure compounds 1 (40 mg) and 2 (100 mg).

The dichloromethane fraction (3.5 g) was chromatographed on column packed with silica (125 g) and was eluted using CH_2Cl_2: MeOH gradient elution to give three main subfractions I, II and III. Subfraction III (0.8 g) was rechromatographed on silica gel column using isocratic elution system of CH_2Cl_2: MeOH (9.5:0.5) which gave Compound 3 (40 mg) purified and crystallized from methanol, and another subfraction (150 mg) that was subjected to Sephadex LH-20 column chromatography using methanol which gave Compound 4 (15 mg).

The ethyl acetate fraction was fractionated by silica gel column chromatography. Elution was performed using gradient system of CH_2Cl_2: MeOH; where six groups (E-I to E-VI) were obtained. Group E-II; CH_2Cl_2: MeOH (90:10) was subjected to silica gel column chromatography using CH_2Cl_2: MeOH in a gradient elution where two subfractions were obtained E-II-a (0.6 g) and E-II-b (1.4 g) eluted with CH_2Cl_2: MeOH (90:10). The subfraction E-II-a was subjected to repeated Sephadex LH-20 column chromatography using methanol to give pure compound 5 (30 mg). Group E-III; CH_2Cl_2: MeOH (80:20) was subjected to silica gel column chromatography using CH_2Cl_2: MeOH in a gradient elution where three subfractions were obtained E-III-a (1.8 g), E-III-b (850 mg) and E-III-c (1.3 g) eluted with CH_2Cl_2: MeOH (85:15). Subfraction E-III-a (1.8 g) was rechromatographed on silica gel column chromatography giving rise to another subfraction E-III-a1 (100 mg) which was subjected to preparative silica gel G60 F254 TLC using system S3 to give pure compound 6 (12 mg). The subfractions E-III-b (850 mg) and E-III-c (1.3 g) were subjected to repeated Sephadex LH-20 column chromatography using methanol to give pure compounds 7 (14 mg) & 8 (10 mg) respectively. Group E-IV; CH_2Cl_2: MeOH (70:30) was subjected to silica gel column chromatography using CH_2Cl_2: MeOH in a gradient elution manner, where three subfractions were obtained E-IV-a (1.4 g), E-IV-b (600 mg) and E-IV-c (900 mg) eluted with CH_2Cl_2: MeOH (80:20). Subfraction E-IV-b (600 mg) was subjected to repeated sephadex LH-20 column chromatography using methanol to give pure compound 9 (14 mg); Also, subfraction E-IV-c (900 mg) was subjected to sephadex LH-20 to give another subfraction E-IV-c1 (90 mg) that was subjected to preparative silica gel G_{60} F_{254} TLC using system S4 to give pure compound 10 (10mg).

Acid hydrolysis

Few milligrams of compounds 6, 7, 8 and 9 were separately dissolved in 5 ml MeOH followed by addition of equal volume of 10% H_2SO_4 v/v. The mixture was allowed to reflux for 3 hrs, then cooled. The hydrolyzate was fractionated with ethyl acetate three times, distilled off and the aglycone was subjected to TLC using CH_2Cl_2: MeOH (90:10) solvent system. The acidic solution was then neutralized with barium carbonate, concentrated and spotted alongside authentic sugars on Whatman No. 1 sheets using n- butanol-acetic acid-water (4:1:2, v/v/v) as a solvent system.

Antimicrobial activity

Test Microorganisms

Bacterial strains included *Staphylococcus aureus* as Gram-positive bacteria and *E coli* as Gram-negative bacteria. fungal strain *Candida albicans* was used. The bacterial and fungal strains were clinically isolated and kindly provided by the infection control research unit (Assiut University Hospital). Strains were grown on Mueller–Hinton agar medium at 37 °C. For preparation of bacterial suspensions single isolated colonies were suspended in sterile Mueller-Hinton broth to obtain the desired concentrations.

A. Antibacterial activity

These strains were grown in Mueller-Hinton broth or Mueller-Hinton agar medium at 37 °C. A single isolated colony of each bacteria was picked from the agar plate culture and suspended in 0.9% (w/v) sterile aqueous saline solution to prepare bacterial suspensions of 0.5 McFarland standard and diluted to give a final concentration of 108 CFU/ml. Antibacterial screening of *C. mitis* leaf extracts was investigated using agar well diffusion method [8, 9]. Bacterial suspensions of either *Staph. aureus* or *E. coli* strains were mixed with Sabouraud agar (20 ml) in sterile petri dishes (9 cm in diameter) and the agar plates were allowed to solidify. After solidification, wells were made in the agar plates using a pore-maker of size 10 mm and filled with 100 µL of different extracts (20 mg/ml). Ampicillin (10µg/ml) and Gentamicin (5µg/ml) were used as positive controls, while DMSO: H2O mixture (1:1 v/v) served as a negative control. Plates were incubated at 37 °C for 24 hrs and the diameters of inhibition zone were measured using a digital caliber. Inhibition zone diameter was an indicator for the antibacterial activity of the studied solutions.

B. Antifungal activity

The antifungal activity of the tested extracts was evaluated by the agar dilution method [10], using Emmon's Sabouraud Dextrose Agar (ESDA) as a growth medium. Stock solutions of the test extracts were prepared at initial concentration of 20 mg/ml in DMSO-Water mixture (1:1 v/v), and the reference standard antifungal drug Clotrimazole was prepared in the same mixture at concentration (5 µg/ml) and incorporated into the growth medium and plates were poured. Plates were inculated with 0.05 ml of the fungal suspension (approximately 5-10 conidia or hyphal element/ml 0.9%

normal saline) and incubated at 30 ºC until macroscopically visible growth appeared in the control (48-96 hrs post inoculation).

Determination of the minimal inhibitory concentration (MIC)

MIC of *C. mitis* leaf extracts were determined against *Staph. aureus* and *E. coli* strains using microtiter broth dilution method [11], according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012). Sterile 96-well rounded bottom plates were used. Samples were vortexed before the experiment to ensure homogenous distribution of the particles. Mueller Hinton broth (100 µL) was dispensed into all wells of the microtiter plate. Each tested extract was added to the first well and 10 serial two-fold dilutions were made (20-0.039 mg/ml). Bacterial suspensions were added into all wells except for control wells. Plates were incubated at 37 °C for 24 hrs and evaluated for the minimum inhibitory concentration by visual examination of the culture turbidity.

Results

Ten compounds were successively isolated from the ethanolic extract of the leaves of *C. mitis*.

Compound 1: obtained as white fine needles (40 mg from acetone), m.p. 198-200 °C, R_f 0.62 (S1). From mixed melting point as well as co-chromatography with authentic samples, it showed the same R_f value and colour reaction of β-amyrin.

Compound 2: obtained as white needles (100 mg from acetone), m.p. 135-137 °C. R_f 0.54 (S1). IR (KBr disc) showed 3460 cm^{-1} broad, assigned for OH stretching, 2960 and 2866 cm^{-1}, for Sp^3 C-H stretching, 1444 and 1370cm^{-1} for geminal dimethyl 1038 cm^{-1} for C-O stretching. From the above data in addition to mixed melting point as well as co-chromatography with authentic samples, it showed the same R_f value and colour reaction of β-sitosterol.

Compound 3: obtained as white powder (40 mg from cold methanol), m.p. 272-274 °C. R_f 0.48 (S2). From the above data in addition to mixed melting point as well as co-chromatography with authentic samples, it showed the same R_f value and colour reaction of β-sitosterol-3-O-β-D glucoside.

Compound 4: obtained as yellow powder (15 mg), m.p. 276-278 °C. R_f 0.51 (S2). UV (MeOH): λmax 264, 369; +NaOMe: 265, 415; +$AlCl_3$: 267, 413; +$AlCl_3$/HCl: 266, 413; +NaOAc: 278, 377; +NaOAc/H_3BO_3: 264, 370 nm; ^{1}HNMR ($CDCl_3$, 400 MHz): δH 6.21 (1H, *d*, J= 2.0 Hz, H-6), 6.35 (1H, *d*, J= 1.6 Hz, H-8), 6.89 (2H, *d*, J= 8.8 Hz, H-3',5'), 8.03 (2H, *d*, J= 8.8 Hz, H-2', 6'); EI-MS: $M^{\bullet+}$ = 286 m/z. By co-chromatography with authentic sample and comparison with the reported data [12], compound 4 was identified as kaempferol.

Compound 5: obtained as yellow powder (30 mg), m.p. 316-318 °C. R_f 0.47 (S2). UV (MeOH): λmax 266, 373; +NaOMe: 270, 424; +$AlCl_3$: 281, 417; +$AlCl_3$/HCl: 279, 393; +NaOAc: 275, 389; +NaOAc/H_3BO_3: 267, 398 nm; ^{1}HNMR (DMSO, d_6, 400 MHz): δH 6.18 (1H, *d*, J= 1.6 Hz, H-6), 6.40 (1H, *d*, J= 1.6 Hz, H-8), 6.90 (1H, *d*, J= 8.4 Hz, H-5'), 7.55 (1H, *dd*, J= 8.4 Hz, H- 6'), 7.68 (1H, *d*, J= 1.6 Hz, H-2'); EI-MS: $M^{\bullet+}$ = 302 m/z. By co-chromatography with authentic sample and comparison with the reported data [12], compound 5 was identified as quercetin.

Compound 6: obtained as yellow powder (19 mg), m.p. 176 °C. R_f 0.47 (S3). UV (MeOH): λmax 263, 357; +NaOMe: 264, 409; +$AlCl_3$: 277, 416; +$AlCl_3$/HCl: 275, 415; +NaOAc: 275, 380; +NaOAc/H_3BO_3: 264, 359 nm; ^{1}HNMR (DMSO-*d6*, 400 MHz): δH 3.18-3.59 (5H, *m*, sugar protons), 5.45 (1H, *d*, J=7.2 Hz, H-1''), 6.12 (1H, *brs*, H-6), 6.34 (1H, *brs*, H-8), 6.89 (2H, *d*, J= 8.8 Hz, H-3', 5'), 8.04 (2H, *d*, J=8.4 Hz, H- 2', 6'), , 12.7 (1H, *s*, 5-OH); ^{13}C-NMR (DMSO-*d6*, 100 MHz): δC 156.27 (C-2), 133.51 (C-3), 177.54 (C-4), 161.59 (C-5), 99.78 (C-6), 167.04 (C-7), 94.42 (C-8), 157.06 (C-9), 103.57 (C-10), 121.38 (C-1'), 131.27 (C-2', C-6'), 115.57 (C-3', C-6'), 160.46 (C- 4'), 101.53 (C-1''), 74.70 (C-2''), 77.92 (C-3''), 70.34 (C-4''), 76.91 (C-5''), 61.30 (C-6''). By co chromatography with authentic sample and comparison with the reported data [12, 13], compound 6 was identified as kaempferol-3-O-β-D-glucopyranoside known as astragalin.

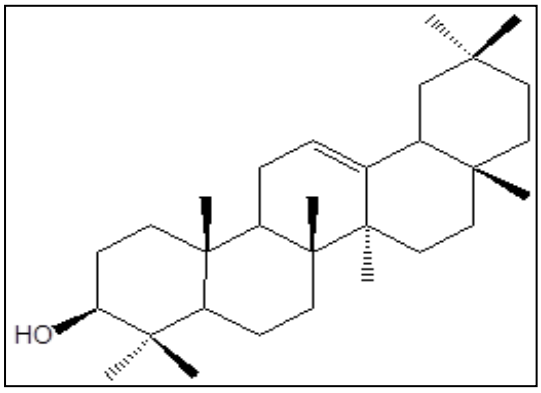
Compound 7: obtained as yellow powder (25 mg), m.p. 240 °C. R_f 0.44 (S3). UV (MeOH): λmax 263, 358; +NaOMe: 275, 416; +$AlCl_3$: 279, 410; +$AlCl_3$/HCl: 277, 383; +NaOAc: 279, 369; +NaOAc/H_3BO_3: 265, 379 nm; ^{1}HNMR (DMSO-*d6*, 400 MHz): δH 3.09-3.60 (5H, *m*, sugar protons), 5.48 (1H, *d*, J=6.8 Hz, H-1''), 6.21 (1H, *brs*, H-6), 6.41 (1H, *brs*, H-8), 6.86 (1H, *d*, J= 8.8 Hz, 5'), 7.58 (1H, *dd*, J=8.0, 2.2 Hz, H- 6'), 7.59 (1H, *d*, J= 2.2 Hz, H- 2'), 12.9 (1H, *s*, 5-OH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δC 156.62 (C-2), 133.77 (C-3), 177.89 (C-4), 161.70 (C-5), 99.14 (C-6), 164.68 (C-7), 93.98 (C-8), 156.79 (C-9), 104.41 (C-10), 121.62 (C-1'), 115.67 (C-2''), 146.28 (C-3'), 148.93 (C-4'), 116.66 (C- 5'), 122.06 (C-6'), 101.32 (C-1''), 74.96 (C-2''), 76.96 (C-3''), 70.39 (C-4''), 78.03 (C-5''), 61.43 (C-6''). By co chromatography with authentic sample and comparison with the reported data [13, 14], compound 7 was identified as quercetin-3-O-β-D-glucopyranoside known as isoquercetrin.

Compound 8: obtained as yellow powder (14 mg), m.p. 200 °C. R_f 0.65 (S4). UV (MeOH): λmax 265, 356; +NaOMe: 279, 413; +$AlCl_3$: 281, 405; +$AlCl_3$/HCl: 280, 405; +NaOAc: 278, 366; +NaOAc/H_3BO_3: 265, 359 nm; ^{1}HNMR (DMSO-*d6*, 400 MHz): δH 3.07-3.68 (9H, *m*, sugars protons), 1.00 (3H, *d*, J= 5.6 Hz, H-6'''), 5.32 (1H, *d*, J=7.2 Hz, H-1''), 4.39 (1H, *brs*, H-1'''), 6.21 (1H, *brs*, H-6), 6.41 (1H, *brs*, H-8), 6.90 (2H, *d*, J= 8.4 Hz, H-3', 5'), 8.00 (2H, *d*, J=8.4 Hz, H- 2', 6'), 12.77 (1H, *s*, 5-OH); ^{13}C-NMR (DMSO-*d6*, 100 MHz): δC 156.97 (C-2), 133.67 (C-3), 177.81 (C-4), 161.65 (C-5), 99.78 (C-6), 164.83 (C-7), 94.42 (C-8), 157.28 (C-9), 104.37 (C-10), 121.35 (C-1'), 131.34 (C-2', C-6'), 115.56 (C-3', C-6'), 160.36 (C- 4'), 101.82 (C-1''), 74.62 (C-2''), 76.82 (C-3''), 70.38 (C-4''), 76.20 (C-5''), 68.71 (C-6''), 101.23 (C-1'''), 70.81 (C-2'''), 71.06 (C-3'''), 72.2 (C-4'''), 67.35 (C-5'''), 18.19 (C-6'''). By co chromatography with authentic sample and comparison with the reported data [13, 15], compound 8 was identified as kaempferol-3-O-rutinoside known as nicotiflorin.

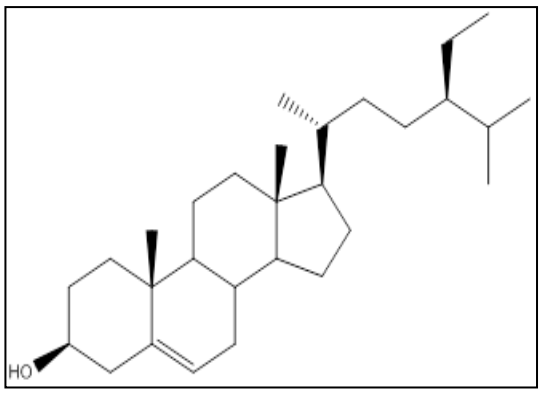
Compound 9: obtained as yellow powder (10 mg), m.p. 125 °C. R_f 0.62 (S4). UV (MeOH): λmax 268, 356; +NaOMe: 274, 417; +$AlCl_3$: 279, 415; +$AlCl_3$/HCl: 278, 384; +NaOAc: 281, 378; +NaOAc/H_3BO_3: 269, 381 nm; ^{1}HNMR (DMSO-*d6*, 400 MHz): δH 3.06-3.70 (9H, *m*, sugars protons), 1.01 (3H, *d*, J= 6.4 Hz, H-6'''), 5.31 (1H, *d*, J=6.4 Hz, H-1''), 4.39 (1H, *brs*, H-1'''), 6.12 (1H, *brs*, H-6), 6.31 (1H, *brs*, H-8), 6.83 (1H, *d*, J= 8.4 Hz, 5'), 7.53 (1H, *dd*, J=8.4, 2.2 Hz, H- 6'), 7.54 (1H, *d*, J= 2.2 Hz, H- 2'),, 13.05 (1H, *s*, 5-OH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δC 156.87 (C-2), 133.64 (C-3), 177.47 (C-4), 161.56 (C-5), 99.57 (C-6), 163.48 (C-7), 94.30

(C-8), 157.00 (C-9), 103.78 (C-10), 121.35 (C-1'), 115.67 (C-2'), 145.32 (C-3'), 149.12 (C- 4'), 116.49 (C-5'), 122.09 (C-6'), 101.92 (C-1''), 74.54 (C-2''), 76.91 (C-3''), 70.42 (C-4''), 76.31 (C-5''), 67.47 (C-6''), 101.22 (C-1'''), 70.81 (C-2'''), 71.01 (C-3'''), 72.31 (C-4'''), 68.70 (C-5'''), 18.19 (C-6'''). By co chromatography with authentic sample and comparison with the reported data [13, 16], compound 9 was identified as quercetin-3-O-rutinoside known as rutin.

Compound 10: obtained as an oily residue (12 mg), soluble in methanol and insoluble in n-hexane and chloroform. ^{1}HNMR (DMSO-d_6, 400 MHz): δH 1.77-1.92 (2H, *m*, H-2), 3.89 (1H, *brs*, H-3), 3.62 (1H, *m*, H-4), 5.02 (1H, *brs*, H-5), 2.11 (2H, *m*, H-6), 3.57 (3H, *s*, -OCH_3), 7.03 (1H, *brs*, H-2'), 6.78 (1H, *brs*, H-5'), 6.98 (1H, *d*, J= 8 Hz, H-6'), 7.41 (1H, *d*, J=16 Hz, H-7'), 6.13 (1H, *d*, J=16 Hz, H-8'). ^{13}C-NMR (DMSO-d_6, 100 MHz): δC 73.5 (C-1), 35.54 (C-2), 67.31 (C-3), 71.48 (C-4), 69.78 (C-5), 37.69 (C-6), 174.09 (C-7), 52.27 (-OCH_3), 125.67 (C-1'), 115.02 (C-2'), 145.67 (C-3'), 149.26 (C-4'), 116.35 (C- 5'), 121.85 (C-6'), 146.23 (C-7'), 114.17 (C-8'), 165.87 (C-9'). By co chromatography with authentic sample and comparison with the reported data [17], compound 10 was identified as chlorogenic acid methyl ester.



Compound 1



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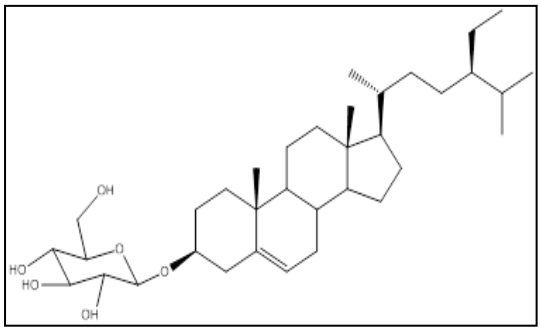

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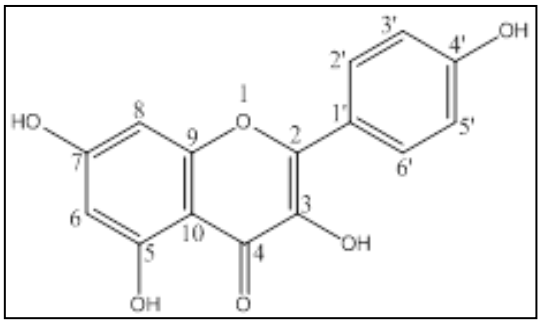

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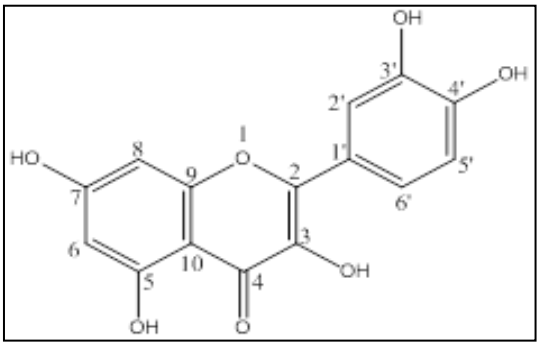

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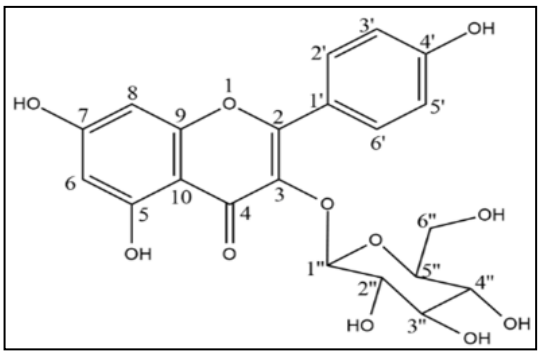
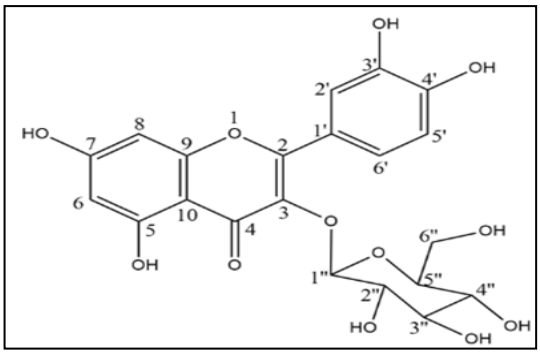


Compound 6



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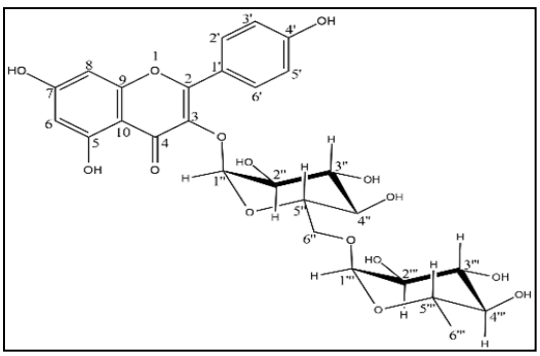
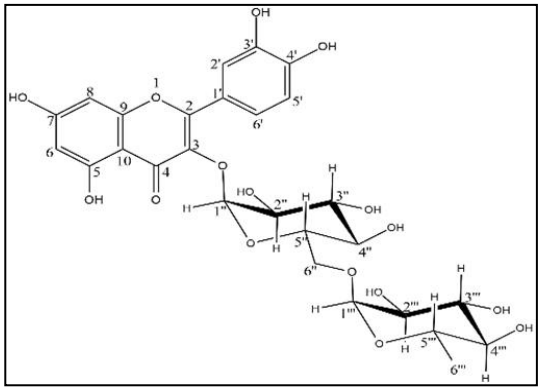
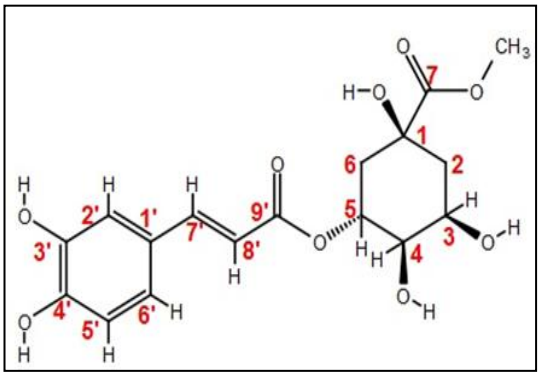


Compound 8



Compound 9



Compound 10

Antimicrobial study

The results including the inhibition zone diameter and minimal inhibitory concentration of each of the tested extracts are listed in (Table 1).

Table 1: Inhibiton zones diameter (IZD) and MICs (Given in brackets) of the different fractions of the leaves of C. mitis Lour. against the teste d bacterial & fungal strains:

Group	Inhibition zone diameter in mm and MIC (mg/ml)		
	S. aureus	***E. coli***	***C. albicans***
Total extract (20 mg/ml)	8 mm (2.5)	10 mm (5)	
n-Hexane fr. (20 mg/ml)	---	---	---
Chloroform fr. (20 mg/ml)	---	---	---
Ethyl acetate fr. (20 mg/ml)	20 mm (2.5)	11 mm (5)	---
n-Butanol fr. (20 mg/ml)	17 mm (2.5)	14 mm (5)	12 mm (5)
Aqueous fr. (20 mg/ml)	7 mm (2.5)	7 mm (5)	12 mm (5)
Controls			
Gentamicin (5µg/ml)	---	26 mm (0.12)	---
Ampicillin (10µg/ml)	28 mm (0.24)	---	---
Clotrimazole (5µg/ml)	---	---	24 mm (0.24)
Negative control (DMSO:Water)	---	---	---

Discussion

Phytochemical analysis of the leaves of *C. mitis* Lour. resulted in isolation and identification of ten compounds; two of them are firstly reported in the family Arecaceae (kaempferol-3-O-rutinoside and chlorogenic acid methyl ester), six compounds are firstly reported in the plant (β-amyrin, β-sitosterol, β-sitosterol-3-O-β-D glucoside, kaempferol, quercetin, kaempferol-3-O-β-D-glucopyranoside) and two compounds previously isolated from the plant (quercetin-3-O-β-D-glucopyranoside, quercetin-3-Orutinoside).

All the tested bacterial and fungal strains showed different susceptibility to each extract. The *n*-hexane and dichloromethane fractions demonstrated weak activity against the tested strains with a zone of inhibition ranging from 3-5 mm. The total and aqueous fractions exhibited moderate bactericidal activity against both *S. aureus* and *E. coli* relative to the positive controls and caused inhibition zones diameter of 7-10 mm diameters (MIC; 2.5 and 5 mg/ml). The ethyl acetate fraction displayed the strongest bactericidal activity against *S. aureus*, relative to the positive control (Ampicillin) and caused inhibition zone of 20 mm in diameter (MIC; 2.5 mg/ml), and also showed moderate activity against *E. coli* relative to control (Gentamycin) and caused zone of inhibition of 11 mm in diameter. The *n*-butanol fraction demonstrated strong bactericidal activity against both *S. aureus* and *E. coli* relative to positive controls and exhibited inhibition zones diameters of 17-19 mm (MIC; 2.5 mg/ ml). The results of the antifungal study against *C. albicans* strains revealed that the *n*-butanol and aqueous fractions possess a significant antifungal activity with zone of inhibition of 12 mm diameter (MIC; 5 mg/ml), while the other extracts exhibited no antifungal activity. The antimicrobial activities of the total extract and the different fractions of the leaves of *C. mitis* may be attributed to their high flavonoids, saponins and alkaloids contents [18].

Conclusion

Ten compounds were isolated and identified from the leaves extracts of *C. mitis* Lour.by different spectroscopic methods, including sterols, triterpenes, phenolic acids, flavonoids and flavonoids glycosides. Antimicrobial assay for the different extracts and fractions revealed strong antibacterial activities on *staph. aureus* more than *E. coli*; in addition to moderate antifungal activity of n-butanol and aqueous fractions against *candida albicans*.

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