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UPLC/Tandem mass and antimicrobial activity of *Khaya senegalensis* (A.Juss.)

Title in Spanish: *UPLC/Tándem masa y la actividad antimicrobiana de Khaya senegalensis*

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ABSTRACT: Characterization of flavonoids and limonoids in the defatted acetone extract of *Khaya senegalensis* flowers (A. Juss.) contents was performed using ultra performance liquid chromatography (UPLC) coupled with ultraviolet (UV) and electrospray ionization (ESI) mass spectrometry, furthermore, tandem mass spectrometry (MS/MS) was performed to assist in the structural elucidation. The antimicrobial effect was tested against representative gram positive and negative bacteria and candida. Cytotoxicity of extract was evaluated using the mitochondrial-dependent reduction of MTT. The method used enabled identification of five flavonoid glycosides (di and mono-sugar) and twelve limonoids of different types *viz*: mexicanolides, phragmalins and angolensate were tentatively identified. The extract was effective against tested microorganism revealing potent growth inhibitory effect on *Salmonella typhimurium* ATCC 25566, *Escherichia coli* NRRN 3008 , *Pseudomonas aeruginosa* ATCC 10145 and fungus *Candida albicans* EMCC105, MIC ≤ 25µg/µl while MIC ≤ 50 µg/µl for *Bacillus cereus*, *staphylococcus aureus* ATCC 6538. Extract showed cytotoxicity against MCF7 (Breast carcinoma cell line) compared to doxorubicin, IC_{50}=88.1(µg/mL) but no activity on HCT 116 (Colon carcinoma cell line) and HepG2 (liver cell carcinoma) was observed. Bioactive compounds in *K senegalensis* flowers acetone extract possesses promising antimicrobial activity with low cytotoxic effect warrants further investigation for their therapeutic and prophylactic roles.

RESUMEN: Se ha procedido a la caracterización de los flavonoides y limonoides del extracto acetónico de las flores de la especie *Khaya senegalensis* (A. Juss.) El análisis de sus componentes se realizó mediante cromatografía líquida de ultra resolución (UPLC) con detección ultravioleta (UV) y de espectrometría de masas de ionización por electrospray (ESI), así como espectrometría de masas (MS / MS) para ayudar en la elucidación estructural de los compuestos. Se comprobó el efecto antimicrobiano en bacterias gram positivas y negativas y levaduras como el genero *Candida*. La citotoxicidad del extracto se evaluó mediante la reducción mitocondrial dependiente de MTT. El método de análisis permitió la identificación de cinco glucósidos flavonoides (di y mono-azúcar) y doce limonoides de diferentes tipos:se identificaron tentativamente mexicanolidos, phragmalinas y angolensato. El extracto fue efectivo contra microorganismos (≤ MIC 25µg / l), revelando potente efecto inhibidor del crecimiento de *Salmonella typhimurium* (ATCC 25566), *Escherichia coli* (NRRN 3008), *Pseudomonas aeruginosa* (ATCC 10145) y el hongo *Candida albicans* (EMCC105). Igualmente fue eficaz a MIC ≤ 50 mg / l sobre *Bacillus cereus*, Staphylococcus aureus (ATCC 6538). Igualmente, el extracto mostró citotoxicidad contra línea celular de carcinoma de mama (MCF7) en comparación con la doxorrubicina, (IC50 = 88.1 (µg/ mL), pero no modificó la actividad en una línea celular de carcinoma colon (HCT 116) y en células de carcinoma del hígado (HepG2). Los compuestos bioactivos en extracto acetónico de flores de *K. senegalensis* poseen una actividad antimicrobiana prometedora con bajos efectos citotóxicos que garantizan la necesidad de una mayor investigación para conocer completamente sus papeles terapéuticos y profilácticos.

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1. INTRODUCTION

Limonoids exhibited broad biological activities; such as: insect- antifeedant, growth-regulating activity on insects, antimicrobial, anticancer, antiviral and other pharmacological activities. Limonoids have been isolated from several plants; their occurrence in the plant kingdom being abundant in families, Meliaceae and Rutaceae. Those in Rutaceae are structurally less complicated, being

generally limited to the modification of A and B rings. On the other hand, Meliaceae limonoids are characterized by high degree of oxidation and rearrangement exhibited in the parent limonoid structure (1).

Ultra performance liquid chromatography (UPLC) has been one of the important physicochemical methods for identification of constituents in plant extracts, due to its rapidity, sensitivity, and low levels of the sample needed (2).

UPLC–UV analysis usually does not provide sufficient information necessary to distinguish between structural isomers. UPLC equipped with ESI /MS method could be useful in the characterization of these compounds. Data dependent tandem mass spectrometry (MS/MS) was performed to assist in the structural elucidation of both aglycones and glycosidic conjugates, as well as the structures of limonoids.

Anti-microbial has been and will continue to prioritize the health needs in all communities. Particularly, when we encounter the emergence of microbes resistance to antibiotics as result of their overuse. Which makes the search for safe and effective alternatives as essential needs. *Khaya senegalensis*, one of Meliaceae plants, have long been used in folk medicine. Flowers are used in medicines against stomach complaints and syphilis**. (3).** Although, the chemical composition of leaves, bark and seeds have been studied (4, 5), according to our knowledge no phytochemical neither biological studies of flowers were reported. This study attempt to investigate their flavonoids and limonoids constituents as well as antimicrobial and cytotoxic activity.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Acetonitrile and formic acid (LC/MS grade) were obtained from J. T. Baker (Deventer, The Netherlands), milliQ water was used for UPLC analysis. Rutin, isoquercitin, isorhamnetin, kaempferol-3-O-β-D-rutinoside, umbelliferone and kaempferol were obtained from Chromadex (Wesel, Germany). All other chemicals and standards were provided from Sigma-Aldrich (St Louis, MO, USA).Sodium dodecyl sulphate (SDS), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT), Doxrubicin (DOX) (Pharmacia, Belgium).

2.2. Plant material

Khaya senegalensis (A. Juss) flowers were collected during April-May from Giza Zoo, Egypt. Plant specimens are deposited at Faculty of pharmacy-Cairo University (voucher No.: 10-6-2014a).

2.3. Plant extraction

The shadow dried powdered flowers (100g) were defatted with petroleum ether then extracted with acetone, the acetone was evaporated under vacuum till dryness to afford brown residue (20gm).

Acetone fraction of defatted powder of *Khaya senegalensis* (A. Juss) flowers (20 ± 0.06 mg) was extracted with 1.8 mL aq.80% MeOH for 10 hours using an orbital shaker in the dark.1 mg of extract was dissolved in 1 mL 100% MeOH. The extract was spiked with (20 µg) umbelliferon as a standard. After sonification for 2 min., sample was centrifuged at 13000 rpm for 3 minutes to remove insoluble material, supernatant was injected.

2.4. High resolution UPLC-PDA-MS analysis

Chromatographic separations were performed on an Acquity UPLC system (Waters) equipped with a HSS T3 column (100 × 1.0 mm, particle size 1.8 µm; Waters) applying two elution binary gradients at a flow rate of 150 µL min–1: (1) 0 - 1 min, isocratic 95% A (water/formic acid, 99.9/0.1 (v/v)), 5% B (acetonitrile/formic acid, 99.9/0.1 (v/v)); 1 - 16 min, linear from 5 to 95% B; 16 t-18 min, isocratic 95% B; 18 - 20 min, isocratic 5% B. The second binary eluent (2) was composed of ammonium acetate 50 mM buffer adjusted to pH 5(A) and 100% acetonitrile (B) using the same elution gradient as above. The injection volume was 3.1 µL (full loop injection). Eluted compounds were detected from m/z 100 to 1000 using a MicrOTOF-Q hybrid quadrupole time-of-flight mass spectrometer (Bruker Daltonics) equipped with an Apollo II electrospray ion source negative ion mode using the following instrument settings: nebulizer gas, nitrogen, 1.6 bar; dry gas, nitrogen, 6 L min–1, 190°C; capillary,5500 V (+4000 V); end plate offset,500 V; funnel 1 RF, 200 Vpp; funnel 2 RF, 200 Vpp; in-source CID energy, 0 V; hexapole RF, 100 Vpp; quadrupole ion energy,5 eV; collision gas, argon; collision energy, 10 eV; collision RF200/400 Vpp (timing 50/50); transfer time, 70 µs; prepulse storage, 5 µs; pulser frequency, 10 kHz; spectra rate, 3 Hz. Internal mass calibration of each analysis was performed by infusion of 20µL 10 mM lithium formiate in isopropanol/water, 1/1 (v/v), at a gradient time of 18 minutes using a diverter valve. Compounds were characterized by their UV-vis. spectra (220-600 nm), retention times relative to umbelliferone, mass spectra and comparison to our in-house database and reference literature (6).

2.4. Tandem mass spectrometry (MS–MS)

MicrOTOF-Q: Precursor ions were selected and fragmented in the collision cell applying collision energies in the range of 10–30 eV. Argon was used as collision gas. Product ions were detected using the following parameter settings: pulser frequency, 10 kHz; spectra rate, 1.5 Hz. For CID of in-source fragment ions, in-source CID energy was increased from 0 to 100 V. Ion Trap MS: MSn mass spectra were also obtained from a LCQ Deca XP MAX system (ThermoElectron, San Jose, USA) equipped with a ESI source (electrospray voltage 4.0 kV, sheath gas: nitrogen; capillary temperature: 275 °C) in negative ionization modes. The Ion Trap MS system is coupled with the exact Waters UPLC setup and using same elution gradient. The MSn spectra were recorded by using the

following conditions: MS/MS analysis with starting collision-induced dissociation energy of 30 eV, negative ionization mode.

2.5. Antimicrobial assay

The antibacterial and antifungal activities were carried out in the Microbial Department, National Research center, using the disc diffusion method (7). A filter paper sterilized disc saturated with measured quantity (20 µl) of the sample (5mg/ml) (100µg/disc) is placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar) or a fungal medium (potato dextrose agar) which has been seeded with the spore suspension of the test organism. Incubation was at 37°C, 24 h for bacteria and at 28°C, 72 h for fungi. All measurements were done in methanol as a solvent which has zero inhibition activity. The antimicrobial activity of the tested compounds were examined with gram positive bacteria, *Bacillus cereus* and *Staphylococcus aureus* ATCC 6538, and gram negative bacteria *Salmonella typhimurium* ATCC 25566, *Escherichia coli* NRRN 3008 and *Pseudomonas aeruginosa* ATCC 10145 and fungus *Candida albicans* EMCC105.The obtained results are compared with the reference antibiotics 20 µl from 5mg/ml solution Cephradine (Bristol Myers-Squibb) and Ketoconazole (*Janssen Pharmaceutica NV),* (100µg/disc). All these steps were carried out under aseptic conditions. The test was carried out in triplicates. The diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism according the following equation: % inhibition = sample inhibition zone (cm) / plate diameter (cm) x 100). The Minimum Inhibition Concentration (MIC) was determined after incubation of the bacterial strains at 37 °C for 24 h and the fungal strain at 28° C for 72 h in the presence of serial dilutions of the test compounds. Each MIC experiment was repeated three times. The MIC was defined as the lowest concentration at which no visible growth was observed.

2.6. Statistical analysis

The results obtained in all analyses were expressed in mean of three ±SE (standard error). Student's *t*- test using GraphPad QuickCalcs on line Software, Inc. CA, US A. by comparing the inhibition zones of samples to those of antibiotics Statistical significance was set at $P < 0.01$.

2.7. In vitro assessment of cytotoxicity

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan (8). All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for MCF7 (Breast carcinoma cell line) HCT 116 (Colon carcinoma cell line) and HEPG2 (liver cell carcinoma).The media are supplemented with 1% antibiotic-antimycotic mixture (10,000U/ mL Potassium Penicillin, 10,000µg/ml Streptomycin Sulphate and 25µg/mL Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37 ºC under 5% CO2.Cells were batch cultured for 10 days, then seeded at concentration of 104 cells/well in fresh complete growth medium in 96-well microliter plastic plates at 37 ºC for 24 hours under 5% CO2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, (without serum) fresh medium was added and cells were incubated either alone (negative control) or with different concentrations of sample (100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 µg/mL), Doxorubicin was used as reference drug at aforementioned concentration; triplicate being prepared for each individual dose. After 48 hours of incubation, medium was aspirated, 40µL MTT salt (2.5µg/mL) were added to each well and incubated for further four hours at 37ºC under 5% CO2. To stop the reaction and dissolving the formed crystals, 200 µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. A positive control which composed of 100µg/mL was used as a known cytotoxic natural agent (*Annona cherimola* leaves) who gives 100% lethality under the same conditions (9). The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at λ 595 nm and a reference wavelength at λ 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula: (1- (Reading of extract / Reading of negative control)) x 100.

3. RESULTS

3.1. UPLC of acetone extract

The UPLC profile for monitoring the separation process is illustrated in (Figure 1) the compounds tentatively identified from mass are compiled in (Table 1). The elution order followed a sequence of decreasing polarity, whereby flavonoid diglucosides eluted first, followed by monoglucosides then free aglycones and highly oxygenated triterpenoids.

3.2. Antimicrobial effect of acetone extract

As shown in Table 2 the tested extract is clearly active against gram negative bacteria represented by *Escherichia coli* and *Salmonella typhimurium* reaching 100% the activity of the reference antibiotic followed by the gram negative bacterium *Pseudomonas aeruginosa* (90%). At the same time our extract exhibited antimicrobial activity against gram positive bacteria *Bacillus cereus* and *Staphylococcus aureus* with inhibitory effect of two thirds the reference antibiotic. With respect to *Candida albicans* the tested extract exerts mild activity against this pathogen. These results are of great value to biological applications

thus they encouraged us to study the minimal inhibitory concentration (MIC) of the extract against each microbial organism (Table 2). The obtained results revealed that MIC of the tested extract was about 5 µ L (25µg) per disc against gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium,* in addition to the yeast pathogen *Candida albicans*. Meanwhile, MIC against gram positive bacteria *Bacillus cereus* and *Staphylococcus aureus* ranged 5 µ L (25µg) ≥10 µL (50 µg) per disc. These results confirm the stronger inhibitory effect of the extract against gram negative bacteria as the smaller the MIC the stronger antimicrobial effect.

3.3. Cytotoxicity of acetone extract

The acetone extract was tested *in-vitro* on human carcinoma cell line (HepG2 – MCF7 – HCT 116). The concentration of extract that is required for 50% inhibition in cell viability (IC_{50}) against MCF7 was IC_{50} =88.1 (µg/mL), compared to Doxorubicin IC_{50} = 26.1 (µg/mL), but neither HEPG2 nor HCT 116 cell lines were inhibited at tested concentrations.

4. DISCUSSION

Coupling of liquid chromatography (LC) with ultraviolet (UV) and electrospray ionization (ESI) mass spectrometric has been demonstrated as a powerful tool for the identification of compounds in plant extracts (10). The method described in our study allowed identification of seventeen compounds from *Khaya senegalensis* flowers.

Flavonoids

UV absorption of flavonoids shows the two characteristic maxima in the ranges λ_{max}240-285 nm (band II) and 300-370 (band I) (11).

Five flavonoid glycosides (di and mono-sugar) were identified. Quercetin glycosides were isolated before from *Khaya senegalensis* (A. Juss.) leaves (12), while this is the first report of luteolin and isorhamnetin glycosides in this plant. The neohesperidoside sugar moiety (rhamnoglucoside 1 ⟶2)) in compound 1 is concluded from fragment at m/z 505 $(M-H-120)^-$ characteristic for the neohesperidoside sugar rather than rutinoside (rhamnoglucoside (1 ⟶6)) (13). UV absorption of compound 1 at λ_{max} 370 nm (band I) indicates presence of free 3-OH suggesting the attachment of sugar moiety at 7-*O*-. On the other hand, compounds 2, 3 and 5 showed band I UV absorption at λ_{max} range 350-355 nm indicating substituted 3- OH flavonol. So they were identified as isorhamnetin-3-O neohesperidoside, quercetin-3-*O*-rutinoside and quercetin-3-*O*- rhamnoside, respectively.

Peaks at *m/z* 479 and *m/z* 463 for isorhamnetin neohesperidoside and rutin, respectively, were due to sequential loss of terminal sugar moiety (rhamnose) before loss of the other moiety (glucose) and give base peak at *m/z* 315 and *m/z* 301 representing the two aglycones respectively.

Limonoids

Furthermore, 12 limonoids were tentatively identified, *viz*: mexicanolides, phragmalins (as khayanolides) and angolensate, based on exact masses, fragmentation pathways, elemental constituents and phytochemical dictionary of natural products database. The UV absorptions at λmax 250-290 nm are due to the transition interaction between two chromophores of the unsaturated lactone and the furan ring in limonoid compounds (5). A common feature was observed, in the mass fragmentation of limonoids, which are characterized by the loss of a major fragment *m/z* 44 to give a base peak referred to $(M-H-CO_2)^-$ resulting from ring cleavages of cyclic anhydride, other common fragments were *m/z* $(M-H-60)^-$ and $(M-H-88)^-$ resulting from loss of $C_2O_2H_4$ and $C_3O_3H_4$, respectively. Mexicanolide type limonoid had characteristic fragment *m/z* $(M-H-28)^-$, due to the loss of intramolecular carbonyl group.

Compounds were identified for the first time in *K. senegalensis* flowers Table (1); amongst them, some were previously reported from other organs of *K. senegalensis* such as pentahydroxy-oxo-tricyclomeliacate and khayanone (mexicanolide type) have been reported before from stem bark (14,15), while the acetyl derivative of the former in addition to khayanolides and khayseneganins (phragmalin type), have been previously isolated from stem bark, leaves and twigs (5,16), furthermore, 2-hydroxymexicanolide and destigloylswietenin have been isolated from *K. senegalensis* seeds (17). On the other hand, 6, 8-dihydroxycarapin have been isolated before from *K. anthotheca* (18).

Acetone extract of *K. senegalensis* flowers showed moderate activity against MCF7 (Breast carcinoma cell line) compared to doxorubicin, with no activity on HCT 116 (Colon carcinoma cell line) and HEPG2 (liver cell carcinoma). The results implied a selective cytotoxicity of *K. senegalensis* (A.Juss.) flowers acetone extract , which is in accordance with that reported for limonoids from *Citrus reticulata* (19). However , *Khaya senegalensis* Bark Extract was reported to inhibit growth of HT-29, HCT-15 and HCA-7 Colorectal cancer cells with IC_{50} (1.00 , 0.30 , 0.22 µg/µl) respectively (20). Moreover, *K. senegalensis* flowers oil displayed cytotoxic activity against HepG-2, MCF-7 and HCT-116 cell lines with LC_{50} (61.1, 79.7, 61 ppm) respectively (21).

Adebayo and Osman, (22) tested the antimicrobial effect of 200 mg/ml *Khaya Senegalensis* ethanol stem bark extract against *Escherichia coli* and *Salmonella typhi*, they found the zone of inhibition = 20.10 and 17.10 mm respectively. Preceding study reported the MIC values were ranged from 390.63 to 1560 µg/mL for *Khaya senegalensis* bark aqueous acetone extract (23). In fact, a deferential cytotoxic and antimicrobial effect of flavonoids and limonoids depend on several factors: Their chemical structure, concentration, and also on the type of cancer cell lines or the microorganisms revealing somewhat different sensitivity toward different compounds (1,24- 26).

5. CONCLUSION

This is the first report of flavonoids and limonoids

from *k. senegalensis* flowers. The method used enabled identification of five flavonoid glycosides and twelve limonoids of different types viz: mexicanolides, phragmalins and angolensate . Pathogens developed resistance to antibiotics is one of very common health problem for human infectious diseases. Being ethnomedicaly used for treating infection such as diarrhea, wounds and syphilis the results deserve further biochemical studies of extracts from the flowers for their potential medicinal significance.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

7. ACKNOWLEDGMENTS

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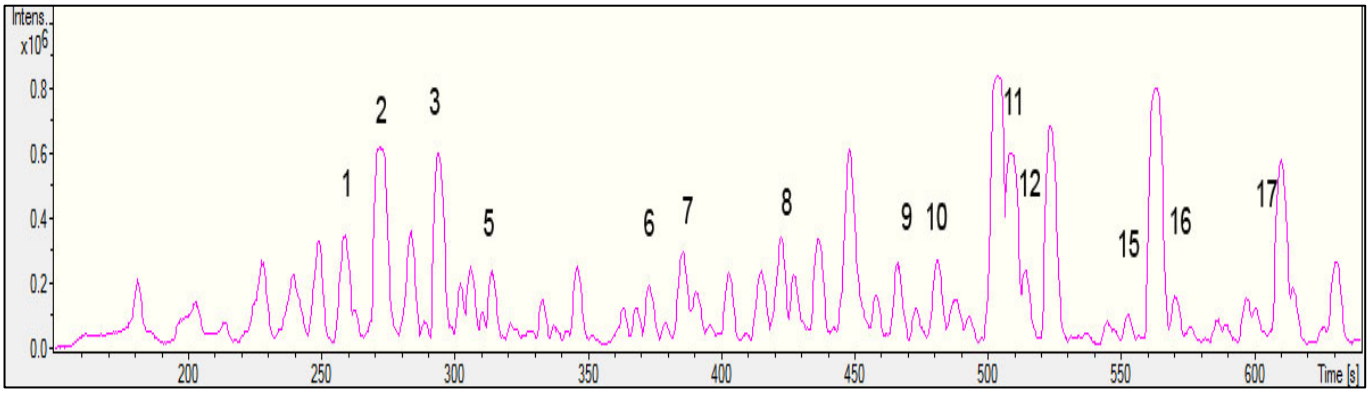

Figure 1. UPLC/ MS chromatogram of *Khaya senegalensis* (A.Juss) flowers acetone fraction.

Table (1): UPLC/ MS-Tandem/Mass of *Khaya senegalensis* (A.Juss.) flowers acetone extract.

NO	Rt. (sec.)	UV (nm)	Mol. ion m/z [M-H]⁻	Element composition	Identified compounds	Error (ppm)	MS/MS
1	247.7	267 305 370	625.1405	$C_{27}H_{29}O_{17}$	Isorhamnetin neohesperidoside	1.0	316 [M-H-neohesperidoside]⁻ 505[M-H-120]⁻
2	272.6	255 265 353	609.1469	$C_{27}H_{29}O_{16}$	Rutin	1.2	301 [M-H-rutinoside]⁻
3	285.0	264, 355	593.1518	$C_{27}H_{29}O_{15}$	Luteolin rutinoside	1.1	285 [M-H-rutinoside]⁻
4	294.2	N.D.	463.0000	$C_{21}H_{19}O_{12}$	Quercetin glucoside	--	301[M-H-glucoside]⁻
5	305.3	256 297 350	447.0932	$C_{21}H_{19}O_{11}$	Quercetin rhamnoside	0.2	301[M-H-rhammoside]⁻
6	368.0	288	515.1921	$C_{27}H_{31}O_{10}$	Khayanolide A.[b]	0.4	471[M-H-CO_2]⁻ 455[M -H-$C_2O_2H_4$]⁻ 427[M-H-$C_3O_3H_4$]⁻

7	390.5	279	533.2024	$C_{27}H_{33}O_{11}$	1, 6, 8, 14,30-Pentahydroxy-3-oxo [3.3.1^{10,2}.1^{1,4}]tricyclomeliac-7-oic acidMethylester. [a]	0.8	515[M-H-H_2O]$^-$ 505[M-CO]$^-$ 487 [M-H-H_2O-CO]$^-$ 445 [M-H- $C_3O_3H_4$]$^-$ 401 [M-H-2H_2O-furanoyl]$^-$
8	415.1	282	559.2182	$C_{29}H_{35}O_{11}$	1- Acetylkhayanolide B.[b]	0.5	516[M-H-C_2OH_3]$^-$ 515[M-H-CO_2]$^-$ 497 [M-H- CO_2-H_2O]$^-$ 471 [M-H- $C_3O_3H_4$]$^-$
9	473.0	284	575.2132	$C_{29}H_{36}O_{12}$	1-acetyl-1,6,8,14,30-Pentahydroxy-3-oxo[3.3.1^{10,2}.1^{1,4}]tricyclomeliac-7-oic acid Methyl ester.[a]	0.6	532[M-H-C_2OH_3]$^-$ 531 [M-H-CO_2]$^-$ 515[M-H-$C_2O_2H_4$]$^-$
10	481.1	278	557.2028	$C_{29}H_{33}O_{11}$	Khayanolide E.[b]	0.8	513[M-H-CO_2]$^-$ 497[M-H- $C_2O_2H_4$]$^-$ 469[M-H- $C_3O_3H_4$]$^-$
11	509.0	280	559.2192	$C_{29}H_{35}O_{11}$	2-Hydroxy, 8α,30α-epoxide, 3-acetyl, detigloylswietenine.[c]	-1.3	515[M-H- CO_2]$^-$ 471 [M-H- $C_3O_3H_4$]$^-$
12	514.4	275	485.2174	$C_{27}H_{33}O_8$	Detigloylswietenine. [c]		467[M-H-H_2O]$^-$ 441[M-H-CO_2]$^-$ 397[M-H- $C_3O_3H_4$]$^-$ 379[M-H- $C_3O_3H_4$ -H_2O]$^-$
13	531.0	N.D.	471.0000	$C_{26}H_{32}O_8$	6-Hydroxy angolensic acid	---	453[M-H-H_2O]$^-$ 427[M-H-C_2H_4O]$^-$ 409[M-H-CO_2-H_2O]$^-$ 331[M-H-CO_2-furan part]$^-$
14	535.0	N.D.	483.0000	$C_{27}H_{32}O_8$	2-hydroxy Mexicanolide	---	439[M-H-CO_2]$^-$
15	537.0	270	547.2174	$C_{28}H_{35}O_{11}$	Khayseneganin F .[b]	2.5	529[M-H- H_2O]$^-$ 487[M-H- $C_2O_2H_4$]$^-$
16	570.0	278	499.1973	$C_{27}H_{31}O_9$	6,8 -Dihydroxycarapin. [c]	0.0	455[M-H-CO_2]$^-$ 411[M-H- $C_3O_3H_4$]$^-$
17	589.5	284	501.2118	$C_{27}H_{33}O_9$	Khayanone (6,8-Dihydroxy, 14,15-dihydrocarapin) [c]	2.4	457 [M-H-CO_2]$^-$

N.D.: Not detected Rt.: Retention time
a: Phragmalin type
b: Rearranged phragmalin type
c: Mexicanolide type

Table (2): The antibacterial and antifungal activities of *Khaya* acetone extract

	Inhibition zone (mm) (% inhibition)		MIC(µg/disc)
Microorganism	**KAE * 100µg/disc**	**Reference drug 100µg/disc**	
Bacillus cereus [a]	19 ± 0.57* [f] (21%)	30 ±0.81 [d] (33%)	≤ 50
Staphylococcus aureus [a]	19.66 ±1.20* [g] (21%)	30 ±0.34[d] (33%)	≤ 50
Salmonella typhimurium[b]	15 ± 1.15 (16%)	15 ±0.47 [d] (16%)	≤ 25
Escherichia coli [b]	20 ± 0.57* [h] (22%)	15 ± 0.40[d] (16%)	≤ 25
Pseudomonas aeruginosa [b]	17 ± 0.57* [i] (18%)	20 ± 0.42 [d] (22%)	≤ 25
Candida albicans [c]	25 ±1.15*[j] (27%)	40 ± 0.33[e] (44%)	≤ 25

* Khaya acetone extract, Each value is mean of three replication of experiment ± SE Values between brackets are the % inhibition compared to control.

a: gram positive bacteria b: gram negative bacteria c: fungus d: Cephradine e: Ketoconazole

Unshared superscript letters between groups are the significant values at (f: P = 0.0001, g: P = 0.0023, h:P = 0.0053, i: P = 0.0147. j: P = 0.0005)

*: Highly significant (P<0.01