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Mohamed R. Elgindi
Department of Pharmacognosy, Faculty of Pharmacy, Egyptian Russian University, Helwan University, Cairo, Egypt.

Abdel Nasser Singab
Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Ibrahim M. Mahmoud
Department of Pharmacognosy, Faculty of Pharmacy, AlAhram Canadian University, Cairo, Egypt.

Sherouk H. Abdullah
Department of Pharmacognosy, Faculty of Pharmacy, Egyptian Russian University, Cairo, Egypt.

Correspondence:
Mohamed R. Elgindi
Department of Pharmacognosy, Faculty of Pharmacy, Egyptian Russian University, Helwan University, Cairo, Egypt.

Phytochemical and biological investigation of the leaves of *Ravenea rivularis* (Arecaceae)

Mohamed R. Elgindi, Abdel Nasser Singab, Ibrahim M. Mahmoud, Sherouk H. Abdullah

Abstract
Background: The main objective of this work is isolation and characterization of chemical compounds with biological studies from the leaves of *Ravenea rivularis* (Arecaceae). Materials and Methods: The air dried plants extracted with70% methanol, n-hexane, ethyl acetate and n-butanol. The compounds were isolated by column chromatography, TLC, PTLC and paper chromatography. The isolated compounds were identified by spectroscopic methods as ^{1}H NMR, UV and MS. Results: The compounds identified are Lupeol acetate, Betulinic acid, Apigenin, Luteolin, luteolin-7-O-β-D-glucopyranoside, ferulic acid, caffeic acid and chlorogenic acid. The leave extracts from *Ravenea rivularis* exhibited antioxidant activity, anti-inflammatory and cytotoxicity against Hep-G2. All those compounds are been reported for the first time in *Ravenea rivularis*.

Keywords: *Ravenea rivularis*, Arecaceae, triterpenoids, flavonoids, Phenolic acid.

1. Introduction
The Arecaceae family is one of the biggest vegetal families of the world and by its morphological aspects is the most characteristic of the tropical flora [1]. This family comprises 1500 species distributed in 200 genera [2]. The chemical composition of the Arecaceae plants includes: diterpenes, triterpenes and their methyl esters, steroids, proantocianidines, flavonoids, saponins and rarely alkaloids [3-7]. The *Ravenea* is a genus of solitaire, dioecious palms belonging to the subfamily Ceroxyloideae, tribe Ceroxyleae. The species *Ravenea rivularis* (also called majesty palm in horticulture) [8] is large palm tree, up to 22 m tall distributed throughout south central Madagascar, Mangoky and Onilahy river systems [9]. The literature reviews not record the phytochemical and biological studies on *Ravenea* rivularis.

2. Material and Methods
2.1.1 Plant Material
Leaves of *Ravenea rivularis* were collected from the Egyptian Orman garden, Giza, Egypt from august 2011. The plant was kindly identified by Agricultural Engineer Terese Labib, El Orman Botanical Garden. The fresh plant leaves were washed with dist. water, completely dried in shade place at room temperature and then powdered by electric mill. The dried powders were kept in a dark place until subjected to the extraction process.

2.1.2 Material for biology
DPPH (1, 1-diphenyl-2-picrylhydrazyl, ascorbic acid, acidified isopropanol**,** MTT {3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide} solution and Hepatocellular Carcinoma (Hep-G2) and Human Breast Adenocarcinoma (MCF-7). Griess reagent, Cells were cultured in RPMI-1640 and Dexamethasone (50mg/ml) as a potent anti-inflammatory.

2.2 Methods
2.2.1 Extraction and Isolation
The powdered leaves of *Ravenea rivularis* (3 Kg) were extracted with 70% methanol at room temperature and the solvent was removed under reduced pressure to give (300 g). The methanolic extract was defatted with petroleum ether. The defatted residue was dissolved in distilled water and the aqueous filtrate was successively extracted with n-hexane followed with ethyl acetate and finally with n-butanol. The three fractions; n-hexane, ethyl acetate and butanol were evaporated until dryness to give 35g, 40g and 4 g respectively. The n-hexane

extract was chromatographed on silica gel column. Elution was achieved with gradient of petroleum ether: ethyl acetate (100% petroleum ether ~ 100% ethyl acetate). Similar fractions were collected. Two major fractions eluted from the column was subjected to silica gel column and sephadex column, followed by PTLC to gave two lupine triterpenoids [10, 11], Lupeol acetate (1) and Betulinic acid (2). The ethyl acetate extract was divided into two portions A and B. Ethyl Acetate extract portion A was fractionated by silica gel column with solvent system ethyl acetate in n-hexane followed by PTLC which afforded three compounds, Apigenin (3), Ferulic acid (4) and Luteolin (5). Ethyl Acetate extract portion B was fractionated by silica gel column with solvent system methanol in chloroform, followed by PPC which afforded two compounds, Luteolin-7-O-β-D-glucoside (6) and Caffeic acid (7). N-Butanol extract was fractionated by silica gel column with solvent system methanol in chloroform followed by PPC which afford one phenolic acid compound, Chlorogenic acid (8).

Compound (1): White needle crystals. EI/MS m/z 468 [M^+] (17.2%). ^{1}H-NMR (400 MHz, $CDCl_3$) at δ: 1.66 (3H, s, H-30), 1.43 (3H, s, H-25) 1.21 (3H, s, H-28), 1.01 (3H, s, H-23), 0.95 (3H, s, H-24), 0.87 (3H, s, H-26), 0.81 (3H, s, H-27), δ 4.50 and 4.61 (2H, s, H-29a and H-29b), and the acetate methyl at δ 2.065 (3H, H-2'), and δ 4.46 (1H, dd, J=4.4, 12.8 Hz, H-3).^{13}C-NMR (100 MHz, $CDCl_3$) at δ: 38.57(C-1), δ 27.53(C-2), δ 81.19(C-3), δ 40.19(C-4), δ 55.57(C-5), δ 18.39(C-6), δ 34.39(C-7), δ 41.04(C-8), δ 50.53(C-9), δ 37.27(C-10), δ 21.13(C-11), δ 25.28(C-12), δ 38.23(C-13), δ 43.02(C-14), δ 27.53(C-15),δ 35.76(C-16), δ 43.19(C-17), δ 48.47(C-18), δ 48.20(C-19), δ 151.21(C-20), δ 29.98(C-21),δ 40.19(C-22), δ 28.14(C-23), δ 16.69(C-24), δ 16.38(C-25), δ 16.16(C-26), δ 14.70(C-27), δ 18.19(C-28), δ 109.54(C-29), δ 19.54(C-30), δ 171.26(C-1'), δ 28.14(C-2').

Compound (2): White powders. EI-MS m/z (rel. Int.): m/z [M^+] 456 (5%). The ^{1}H and ^{13}C NMR spectra of triterpenoid (2) were closely resembled to those of triterpenoid (1). In ^{1}H NMR spectrum (400 MHz, $CDCl_3$): the tertiary methyl appears singlet's at δ 0.75 (H_3-24), 0.82 (H_3-25), 0.94 (H_3-26), 0.97 (H_3-23) and 0.98 (H_3-27). In addition, δ 1.71 (3H, s, H-30), δ 4.62, δ 4.76 (2H, brs, H-29) and δ 3.2 (1H, dd, *J*=5.4, 10.8 Hz, H-3). The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) at δ: 38.87(C-1), δ 26.09(C-2), δ 78.36(C-3), δ 39.08(C-4), δ 55.99(C-5), δ 18.81(C-6), δ 34.89(C-7), δ 41.21(C-8), δ 50.00(C-9), δ 37.68(C-10), δ 21.42(C-11), δ 28.30(C-12), δ 39.37(C-13), δ 42.96(C-14), δ 29.27(C-15),δ 32.80(C-16), δ 56.77(C-17), δ 49.15(C-18), δ 47.56(C-19), δ 151.24(C-20), δ 30.2(C-21),δ 37.68(C-22), δ 27.4(C-23), δ 15.24(C-24), δ 15.95(C-25), δ 15.61(C-26), δ 14.92(C-27), δ 179.63(C-28), δ 109.90(C-29), δ 19.645(C-30).

Compound (3): Dark yellow powders. UV (λ_{max} in MeOH): gives bands at 331 and 272 nm for band I and II, addition of NaOMe; 400, 332 and 268, NaOAc; 387, 306 and 281, H_3BO_3; 342and 268, $AlCl_3$; 387, 351, 306 and 279, while HCl; 386, 346, 304 and 279. EI/MS m/z 270 [M^+] (100%). ^{1}H-NMR (400 MHz, DMSO-d_6) at δ: 6.78 *(1H, s, H-3),* 12.97 *(s, 5-OH),* 6.19 *(1H, d, J=1.6 Hz, H-6),* 10.83 *(s, 7-OH),* 6.48 *(1H, d, J=1.6 Hz, H-8),* 7.91 *(1H, d, J=8.8 Hz, H-2'),* 6.92 *(1H, d, J=8.8 Hz, H-3'),* δ 10.35 *(s, 4'-OH),* 6.93 *(1H, d, J=8.8 Hz, H-5'),* 7.93 *(1H, d, J=8.8 Hz, H-6').*^{13}C-NMR (100 MHz, DMSO-d_6) at δ: 163.7 (C-2), δ 102.80 (C-3), δ 181.69 (C-4), δ 161.42 (C-5), δ 98.84 (C-6), δ 164.25 (C-7), δ 93.95 (C-8), δ 157.3 (C-9), δ 103.625 (C-10), δ 121.15 (C-1'), δ 128.42 (C-2'), δ 115.94 (C-3'), δ 161.16 (C-4'), δ 115.94 (C-5'), δ 128.42 (C-6').

Compound (4): Fine powders. EI/MS m/z 194 [M^+] (100%). ^{1}H-NMR (400 MHz, CD_3OD) at δ: 7.18 *(1H, d, J=2.0 Hz, H-2),* δ 9.59 *(OH, S, H-4),* δ 6.81 *(1H, d, J=8.0 Hz, H-5),* δ 7.07 (1H, *dd, J=8.0, 2.0 Hz, H-6),* δ 7.61 (1H, *d, J=16.0 Hz, H-*7), δ 6.31 (1H, *d, J=16.0 Hz, H-*8), δ 9.15 *(1H, S, H-9),* δ 3.95 *(3H, S, O-Me).* ^{13}C-NMR (100 MHz, CD_3OD) at δ: 127.8 (C-1), δ 111.8 (C-2), δ 149.4 (C-3), δ 150.5 (C-4), δ 116.0 (C-5), δ 124.0 (C-6), δ 146.9 (C-7), δ 116.5 (C-8), δ 171.0 (C-9), δ 56.5 (O-Me)**.**

Compound (5): Yellow powders. UV (λ_{max} in MeOH): gives bands at 349 and 253 nm for band I and II, addition of NaOMe; 402, 330 and 265, NaOAc; 390, 325 and 269, H_3BO_3; 430, 370, 301 and 262, $AlCl_3$; 426, 328, 300 and 274, while HCl; 388, 356, 296 and 275. EI/MS m/z 286 [M^+] (75%). ^{1}H-NMR (400 MHz, DMSO-d_6) at δ: 6.67 *(1H, S,H-3),* δ 12.97 *(1H, S, 5-OH),* δ 6.18 *(1H, d, J=2.0 Hz, H-6),* δ 6.44 *(1H, d, J=2.0 Hz, H-8),* δ 7.39 *(1H, m, H-2'),* δ 6.89 *(1H, d, J=8.0 Hz, H-5'),* δ 7.42 *(1H, m, H-6').* ^{13}C-NMR (100 MHz, DMSO-d_6) at δ : 163.87 (C-2), δ 102.85 (C-3), δ 181.62 (C-4), δ 161.46 (C-5), δ 98.82 (C-6), δ 164.14 (C-7), δ 93.82 (C-8), δ 157.28 (C-9), δ 103.67 (C-10), δ 121.49 (C-1'), δ 113.35 (C-2'), δ 145.72 (C-3'), δ 149.69 (C-4'), δ 116.00 (C-5'), δ 118.96 (C-6').

Compound (6): Yellow needles. UV (λ_{max} in MeOH): gives bands at 348 and 255 nm for band I and II, addition of NaOMe; 392, 300 and 262, NaOAc; 405, 364, 266 and 259, H_3BO_3; 370 and 259, $AlCl_3$; 430, 329, 299 and 274, while HCl; 387, 358, 295 and 273. EI/MS m/z 286 [aglycone fragment]. ^{1}H-NMR (400 MHz, DMSO-d_6) at δ: 6.74 *(1H,S, H-3),* δ 12.97 (1H, br, s, H-5), δ 6.43 *(1H, d, J= 2.1 Hz, H-6),* δ 6.78 *(1H, d, J=2.1 Hz, H-8),* δ 7.44 *(1H, d, J=2.1 Hz, H-2'),* 12.97 *(1H, brs, H-3'),* δ 12.97 (1H, brs, H-4'), δ 6.9 (1H, d, J=8.1 Hz, *H-5'),* δ7.42 (1H, dd, J=2.1, 8.1 Hz, *H-6'),* 3.34-5.07 *(6H,m, sugar protons).*^{13}C-NMR (100 MHz, DMSO-d_6) at δ: 164.69 (C-2), δ 103.36 (C-3), δ 182.11 (C-4), δ 161.34 (C-5), δ 100.11 (C-6), δ 163.16 (C-7), δ 94.95 (C-8), δ 157.16 (C-9), δ 105.55 (C-10), δ 121.57 (C-1'), δ 113.75 (C-2'), δ 146.00 (C-3'), δ 150.16 (C-4'), δ 116.20 (C-5'), δ 119.39 (C-6'), δ 99.75 (C-1"), δ 73.33 (C-2"), δ 76.59 (C-3"), δ 69.77 (C-4"), δ 77.36 (C-5"), δ 60.83 (C-6").

Compound (7): Yellow-Brown powders. UV (λ_{max} in MeOH): 292 (3.90), 321 (3.89) nm. EI/MS m/z 180 [M+] (15%). ^{1}H-NMR (400 MHz, CD_3OD) at δ: 7.07 (1H, d, J= 2.0 Hz, H-2), δ 6.82 (1H, d, J= 8.2 Hz, H-5), δ 6.95 (1H, dd, J= 8.2, 2.0 Hz, H-6), δ 7.57 (1H, d, J= 15.9 Hz, H-7), δ 6.25 (1H, d, J= 15.9 Hz, H-8). ^{13}C-NMR (100 MHz, CD_3OD) at δ: 128.29 (C-1), δ 115.69 (C-2), δ 147.16 (C-3), δ 149.84 (C-4), δ 117.05 (C-5), δ 123.38 (C-6), δ 147.58 (C-7), δ 116.0 (C-8), δ 171.63 (C-9).

Compound (8): Buff needles.^{1}H-NMR (400 MHz, D_2O) at δ: 1.77-2.03 *(2H, m, H-2),* δ 5.17 *(1H, m, H-3),* δ 3.66 *(1H, brs, H-4),* δ 3.92 *(1H brs, H-5),* δ 1.77-2.03 *(2H, m, H-6),* δ 7.001 *(1H, d, J=2.0 Hz, H-2'),* δ 9.19 *(1H -OH, brs, H-3'),* δ 9.63 *(1H -OH, s, H-4'),* δ 6.77 *(1H, d, J=8.0 Hz, H-5'),* δ 6.95 *(1H, dd, J=2.0, 8.0 Hz, H-6'),* δ 7.42 *(1H, d, J=15.9 Hz, H-7'),* δ 6.20 *(1H, d, J=15.9 Hz, H-8'),* δ10.998 *(1H, S, COOH).*13C-NMR (100 MHz, D_2O) at δ: 79.2 (C-1), δ 37.55 (C-2), δ 70.99 (C-3), δ 73.14 (C-4), δ 71.4 (C-5), δ 38.68 (C-6), δ 177.01

(COOH), δ 127.83 (C-1'), δ 114.88 (C-2'), δ 144.45 (C-3'), δ 147.92 (C-4'), δ 116.43 (C-5'), δ 122.85 (C-6'), δ 147.28 (C-7'), δ 115.315 (C-8'), δ 169.31 (C-9').

2.2.2. Methods for biology

2.2.2.1 Antioxidant Assay

A 20 µl of different concentrations (0 –125 µg/ml) of tested sample were used, DPPH were incubated for 30 min at 37°.Triplicate wells were prepared for each concentration and the average was calculated. The photometric determination of absorbance at 520 nm was performed by microplate ELISA reader [38].

The half maximal scavenging capacity (SC_{50}) values for each tested sample and ascorbic acid was estimated via dose curve. SC_{50} of each sample was calculated using the curve equation.

2.2.2.2 Anti-inflammatory Assay

Anti-inflammatory assay by Nitric oxide method, in each well of a flat bottom 96 well- microplate, 40 µl freshly prepared Griess reagent was mixed with 40 µl cell supernatant or different concentrations of sodium nitrite ranging from 0-100 µ mole/ml. The plate was incubated for 10 min in the dark and the absorbance of the mixture at 540 nm was determined using the microplate ELISA reader.

A standard curve relating NO in µ mole/ml to the absorbance is constructed, from which the NO level in the cell supernatant is computed by interpolation.

2.2.2.3 Cytotoxic Assay

Cytotoxicity of the total methanolic extract was measured against Hep-G2 and MCF-7 cells using the MTT Cell Viability Assay. Cells ($0.5X10^5$ cells/ well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20 µl of different concentrations of the total extract for 48 h at 37º, in a humidified 5% CO2 atmosphere. After incubation, media were removed and 40 µl MTT solution /well were added and Incubated for an additional 4 hrs. MTT crystals were solubilized by adding 180 µl of acidified isopropanol / well and plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. The method were repeated three times for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability.

Percentage of relative viability was calculated.

3. Results and discussion

3.1 Results and discussion for isolated compounds

The ^{1}H-NMR spectral data of compound 1 showed the presence of eight tertiary methyl singlets at δ: 0.81, 0.87, 0.95, 1.01, 1.21, 1.43, 1.66 and δ 2.065. Two protons appeared at δ 4.50 and δ 4.61 as singlets, representing the exocyclic double bond protons H-29a and H-29b, respectively. A one proton double doublet at δ 4.46 with coupling interactions of 4.4 and 12.8 Hz ascribable to the axial C-3 methine proton. One proton double doublet at δ 0.76 with coupling interaction of 5.8 and 10.8 Hz ascribable to C-5. A three-proton broad signal at δ 2.065 due to acetoxy methyl group. ^{13}C NMR spectrum showed a carbonyl group at δ 171.26, C-3 at δ 81.19 and the alkene carbons at δ 151.21 and δ 109.54. The ^{1}HNMR and ^{13}C-NMR data of compound (1) indicated a pentacyclic triterpenoid of lupane type and comparison of its physical and spectral data with published values confirmed the identity of compound (1) as lupeol acetate [12, 13]. Structure elucidation of this triterpene was published [14] and this compound identified for the first time from the genus *Ravenea*.

The ^{1}H-NMR spectral data of compound 2 showed the presence of five tertiary methyl singlets at δ: 0.75 (H_3-24), 0.82 (H_3-25), 0.94(H_3-26), 0.97 (H_3-23) and δ 0.98 (H_3-27). In addition, the presence of an isopropenyl group was shown in downfield methyl signal at δ 1.71, and two vinylic proton at δ 4.62, δ 4.76 (*br s*). These data indicated that this compound belong to the lupine group. The ^{1}H NMR spectrum further showed a typical lupane H_β-19 proton at δ 3.01 (*m*) and an oxymethine proton at δ 3.22 (*dd, J= 10.8, 5.4* Hz). The large coupling constant between H-3 and H-2 with $J_{ax\text{-}ax} = 10.8$ Hz indicating that 3-hydroxy group was at β–fact [15, 16]. ^{13}C NMR spectrum showed a carbonyl group at δ 179.63, C-3 at δ 78.36 and the alkene carbons at δ 151.24 and δ 109.90. Further information about the compound was obtained from typical EI-Mass related to the fragmentation pattern of lupane type triterpenes through the presence of m/z [M^+] 456, 441 [M^+ - CH_3] (10), 438 [M^+ - H_2O] (20), 426 [M^+ - (15-15)] (10), 415 [M^+ - C_3H_5] (25), 411 [M^+- COOH] (11), 395 (10), 248 [$C_{16}H_{24}O_2$] (10), 220 [$C_{15}H_{24}O$] (40), 203 [220 - OH] (80), 175 [220 - COOH] (10), 207 [$C_{14}H_{23}O$] (30), 189 [207 - H_2O] (90), 205, 207 [M^+ - $C_{16}H_{27}$] (7), 41 (100), Which corresponded to a chemical formula $C_{30}H_{48}O_3$. The ^{1}HNMR and ^{13}C-NMR data of compound (2) indicated a pentacyclic triterpenoid of Lupane type and comparison of its physical and spectral data with published values confirmed the identity of compound (2) [7, 17-21]. Thus on the basis of its spectroscopic data and comparison with the previous report compound (2) was assigned as betulinic acid, Isolated for the first time from the genus *Ravenea*.

UV spectral data in MeOH of compound 3 showed two major absorption bands; band I at 331 nm and band II at 272 nm, which is indicate the presence of a flavone nucleus with no hydroxyl group at position 3 [22-25]. The addition of sodium methoxide resulted in a bathochromic shift (+Δ69 nm) in band I with an increase in intensity, which proved that position 4` has a free OH group [26]. The presence of a shoulder at 332nm in NaOMe along with a bathochromic shift in band II on addition of NaOAc (+Δ9 nm) compared with the same band in MeOH suggested the presence of a free hydroxyl group at C7. On addition of H_3BO_3 to NaOAc the hypochromic shift in band I (-Δ11 nm) suggested the absence of any *ortho*-dihydroxyl groups. The bathochromic shift in band I (+Δ56 nm) on addition of $AlCl_3$; compared with the same band in MeOH, which is still stable even after the addition of HCl indicated the presence of free 5-OH group and confirmed the absence of any *ortho*-dihydroxyl groups. ^{1}H-NMR spectral data showed the aromatic protons of the B-ring as two doublets at δ 7.91, δ 7.93 and δ 6.92, δ 6.93 each doublet with *J*= 8.8 Hz due to *ortho* coupling assigned to H-2`, 6` and H-3`, 5`, respectively; two aromatic protons of the A-ring revealed as two doublet at δ 6.48 and δ 6.19 each proton has *J*=1.6 Hz due to *meta* coupling assigned to H-8 and H-6, respectively. H-3 appeared at δ 6.78 as a single signal [23, 26-28]. The structure of the compound was further supported by ^{13}C NMR spectrum which exhibited a downfield signal at δ 181.67 assignable to C-4. The other downfield signals at δ 164.25 – δ 163.7 - δ 161.16 were due to C-7, C-2 and C-4' to which hydroxyl groups are attached. From the spectral analysis, melting point, and comparing with the published data [26, 29, 30] Compound (3) was identified as Apigenin (Isolated for the first time from the

genus *Ravenea*).

^{1}H-NMR spectral data of compound 4 showed signals at δ ppm, 9.15 (1H, S COOH), two doublets at 7.61 and 6.31 with *J*=16 Hz characteristic of trans olefinic double bond of α, β-unsaturated system, also exhibited one hydroxyl signals at 9.58 and singlet at 3.90 for –OCH_3 also other signals at 7.18, 6.81 and 7.07 with *J*=8.0, 2.0 Hz indicate AB aromatic system. From ^{13}C NMR spectrum, the O-methoxy moiety could be confirmed from signals at 56.5ppm.EI. MS m/z 194 (M^+ 100%), 179 (M^+-15, 25%), 77(C_6H_6, 27%) corresponding to molecular formula $C_{10}H_{20}O_5$ [31]. From the spectral analysis and comparing with the published data [31] compound (4) was identified as Ferulic acid (Isolated for the first time from the genus *Ravenea*).

UV spectral data in MeOH of compound 5 showed two major absorption bands; The UV spectrum of the methanol solution of the compound 5 has two characteristic bands, I at λ = 349 nm, and band II at λ = 253 nm, which indicates that the compound belongs to the group of flavonoids. From the bathochromic shift with $AlCl_3$, i.e. the band I shift from λ = 349 nm, splitting into two bands with peaks at λ = 426 nm and λ = 328 nm, indicated the presence of an OH-group in position 5. After adding the agent NaOAc, the band II shift by 16 nm (269 nm -253 nm), indicated the presence of and OH-group in position 7. When agent NaOAc+H_3BO_3 was added, the shift of band I by 21 nm (370 nm - 349 nm), indicated the presence of OH-group in positions 3 and 4. From the UV spectrum the presence of OH-group in positions 5, 7, 3' and 4' was recorded. The ^{1}H NMR spectral showed the peak in aromatic range between δ 6.18 to δ 7.42. The peak observed at δ 6.67 as singlet was clearly assigned to H – 3 proton. The signals at δ 6.18, 6.44 corresponding to two protons were assignable to protons H-6 and H-8 of ring A. Rest of the aromatic proton signals were of ring B. The compound is tetra substituted in which both their rings A and B are having two hydroxyl groups each. The EI-MS spectral data showed the molecular ion peak as the base peak (M+) at m/z 286 (75%), 273 (22%), 258 (18%), 228 (8%),203 (6%), 165 (3%), 153 (20%), 174 (100%), 148 (70%), 145 (45%), 134 (7%), corresponding to molecular formula $C_{15}H_{10}O_6$.It was further supported by mass spectrum which gave two fragment at m/z 153 [A] and m/z 134 [B] formed due to Retro Diel's Alder fragmentation. The structure of the compound was further supported by ^{13}C NMR spectrum which exhibited a downfield signal at δ 181.62 assignable to C-4. The other downfield signals at δ 161.46 - δ 93.82 – δ 145.72 – δ 149.69 were due to C-5, C-8, C-3′ and C-4′ to which hydroxyl groups are attached. Based on the obtained data above and comparing those data with the published data [22-26, 29, 30, 32] for compound (5) was identified as Luteolin (Isolated for the first time from the genus *Ravenea*).

UV spectral data in MeOH of compound 6 showed two major absorption bands; band I at 348 nm and band II at 255 nm, which is typical of a flavone nucleus with no hydroxyl group at position 3 [22-25], the UV absorbance of methanol solution less than 350 nm indication the presence of flavone nucleous, from the bathochromic shift (+Δ7 nm), and (+Δ44 nm) with NaOMe at band II and band I; confirming the presence of polyhydroxyl groups, the addition of $AlCl_3$ agent, bathochromic shift (+Δ19 nm),indicating presence of hydroxyl group at C-3 or C-5 or ortho-dihdroxy groups, after adding HCl stable UV absorbance was observed in band II; indication presence of hydroxyl group at C-5. While hypsochromic shift (-Δ43 nm) in band I indicating presence of 3', 4' dihydroxyl group, after adding the agent NaOAc bathochromic shift (+Δ57 nm) of band I is ascribable to free 4'-OH group and when agent NaOAc/H_3BO_3 added the bathochromic shift (+Δ22 nm) of band I indicates the presence of 3', 4' dihydroxy group. ^{1}H-NMR spectral data showed characteristic signals of flavone, The ^{1}H-NMR spectral data showed signals for γ-pyrone ring at δ 6.74 (s, H-3), for A-ring atδ6.4 (d, H-6), 6.7 (d, H-8), for B-ring at δ6.9 (d, H-5'), 7.4 (dd, H-6'), 7.4 (d, H-2'). Anomeric proton of glucose appeared at δ5.04 (1H, d). The above data was confirmed by ^{13}C-NMR data. From ^{13}C NMR spectrum, the O-glucosyl moiety could be confirmed from signals of glucose with C-1" at 99.75 ppm, and C-6" at 60.83 ppm. From the spectral analysis, melting point. From the previous data and comparing those data with the published data for luteolin-7-O-glucoside [24, 26, 32, 33], so compound 6was identified as Luteolin-7-O-β-D-glucopyranoside (Isolated for the first time from the genus *Ravenea*).

The ^{1}H-NMR spectra of compound 7 displayed two ortho – coupled doublet (J=8.2 Hz) each for 1H, at δ 6.82 and 6.95 and broad singlet for 1H at δ 7.07 in the aromatic region indicated the presence of a tri-substituted aromatic ring in the molecule. The chemical shifts of these signals indicated the presence of catechol moiety in the molecule, which was confirmed by ^{13}C-NMR chemical shifts of the Hydrogen carrying Carbon atoms at δ 115.69(C-2), 117.05(C-5) and 123.38(C-6).The ^{1}H-NMR spectral data also displayed two doublets (J=15.9 Hz), each for 1H, at δ 7.57 (H-7) and 6.25 (H-8). The large value of coupling constant indicated the presence of Trans – disubstituted ethylene moiety in the molecule. The ^{1}H and ^{13}C chemical shifts of olefinic protons and carbons [δ 147.58(C-7) and 116.0(C-8)] were similar to those of Trans – Cinnamic acid. The ^{13}C-NMR spectral exhibited presence of nine carbon atoms in the molecule. The ^{13}C chemical shifts of a carbon at δ 171.63 indicated the presence of carboxylic functional group in the molecule. The upfield chemical shifts of one of the ethylenic carbon (C-8) and proton (H-1) indicated that the carboxylic group is located at C-8 position. The ^{13}C- chemical shifts of carbon atoms at δ 147.16 (C-3), 149.84 (C-4), indicated that the hydroxyl group are attached at C-3 andC-4 positions. The position of ethylene function was determined by chemical shift of C-1 carbon at δ 128.29 and the downfield chemical shifts of C-7 carbon and H-7 proton of ethylene moiety.).From the previous data and comparing those data with the published data [26, 32], so compound (7) was identified as Caffeic acid (Isolated for the first time from the genus *Ravenea*).

Chlorogenic acid did not show a molecular ion peak but two prominent ion peaks at m/z 180 ($C_9H_8O_4$) and 162 ($C_9H_8O_4$-H_2O) suggesting the presence of caffeoyl moiety.^{1}H-NMR spectra of compound 8 showed signals at δppm 11.0 (singlet, -COOH Carboxyl proton), ^{1}H NMR showed signals at two doublets at δ7.42 and δ 6.20 with J=15.9 Hz characteristic of trans olefinic double bond of α, β-unsaturated system, also exhibited two hydroxyl signals at δ 9.19 and δ 9.63 for H-3' and H-4' and respectively, also displayed two ortho-coupled doublet each for 1H at δ7.001, δ 6.95 and δ6.77 with J=8.0, 2.0 Hz indicate AB aromatic system. The ^{1}H NMR spectra of compound (8) showed the signals of caffeic acid and two methylenes, one oxygen bearing carbon with acid ascribable to cyclo polyoxy carboxylic acid, i.e. quinic acid, respectively. The spectroscopic data were in agreement with those for the structure of caffeoylquinic acid known as chlorogenic acid. The respective compound (8) were identified by comparison of ^{1}H NMR spectroscopic data with those reported in the literature [34, 35]. From ^{13}C NMR spectra, the carboxylic moiety

could be confirmed from signals at δ177.01 ppm. The upfield chemical shifts of one of the ethylenic carbon (C-8) and proton (H-1) indicated that the carboxylic group is located at C-8 position. The ^{13}C NMR chemical shifts of carbon atoms at δ 70.39 (C-3), δ 73.14 (C-4), indicated that the hydroxyl group are attached at C-3 and C-4 position. The position of ethylene function was determined by chemical shift of C-1' at δ 127.83 and the downfield chemical shifts of C-7 proton of ethylene moiety. From the previous data and comparing those data with the published data [26, 32, 36, 37], so compound (8) was identified as Chlorogenic acid (Isolated for the first time from the genus *Ravenea*).

3.2 Results and discussion for Biological activities

3.2.1 Antioxidant activity

The free radical scavenging activity of each extract was determined by using a stable (DPPH) according to the procedure described [38]. The scavenging activity (antioxidant activity) of each extract was expressed as SC_{50} which is defined as the concentration of extract required for 50 % scavenging of DPPH radicals compared with that of ascorbic acid which was used as the standard. The lower SC_{50} value corresponds to higher scavenging activity (higher antioxidant activity) of plant extract. The tested samples possessed antioxidant scavenging affinity against DPPH radicals, the SC_{50} for the samples; ethyl acetate, total methanol and n-hexane extracts were 251, 329.5 and 306 µg/ml respectively, compared with our standard Ascorbic acid of SC_{50} 15.25 µg/ml as shown in figure 2.

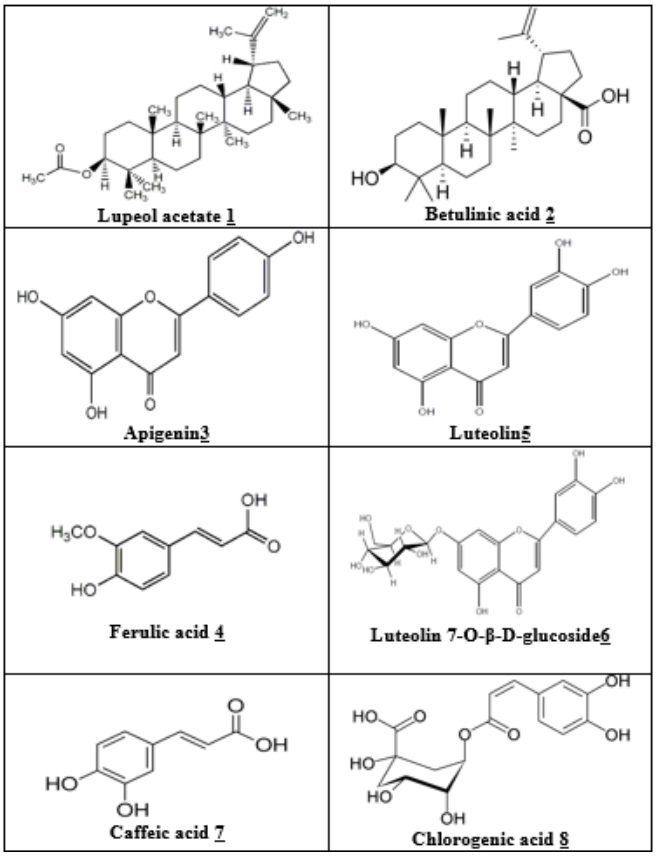

Fig 1: Structure of elucidated compounds

3.2.2 Anti-inflammatory activity

Using Nitric oxide method [39], the results indicated that the inflammagen lipopolysaccharide (LPS 100 µg/ml) induced nitric oxide production up to 3.7 folds of the control as in figure 3, while that the potent anti-inflammatory Dexamethasone (50 ng/ml) inhibited nitric oxide production to 3.36 µ mole/ml compared to 9.48 µ mole/ml of that of the LPS with level of 65% inhibition. Methanolic extract (125 µg/ml); showed anti- inflammatory effect (66 % inhibition) in comparison with Dexamethasone (65 % inhibition) as shown in figure 4.

3.2.3 Cytotoxic activity

Using MTT assay, the effect of the extract on the proliferation ofMCF-7 cells and Hep-G2 was studied after 48 h of incubation. The treatment with Total extract showed strong cytotoxic effect against MCF-7with determined IC_{50} value to be 70.85 µg/ml as shown in figure 5,on the other hand the total extract showed no significant cytotoxic effect on Hep-G2 with increase in cell proliferation by increasing sample concentration as in figure 6 [40].

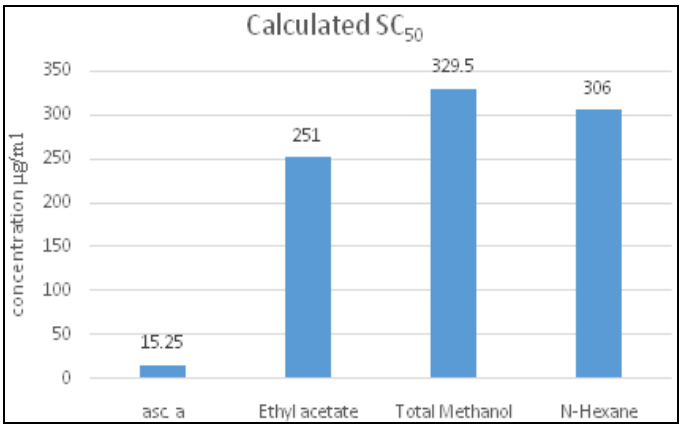

Fig 2: Antioxidant activity against DPPH radicals (Calculated Sc 50 for ascorbic acid and the tested samples).

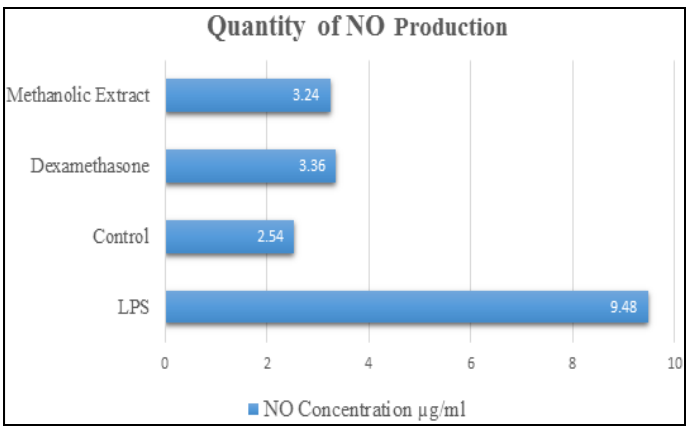

Fig 3: *In-Vitro* Anti-inflammatory by Nitric oxide method, data expressed as Quantity of nitric oxide produced of both the sample and Dexamethasone compared to the LPS- stimulated cells alone.

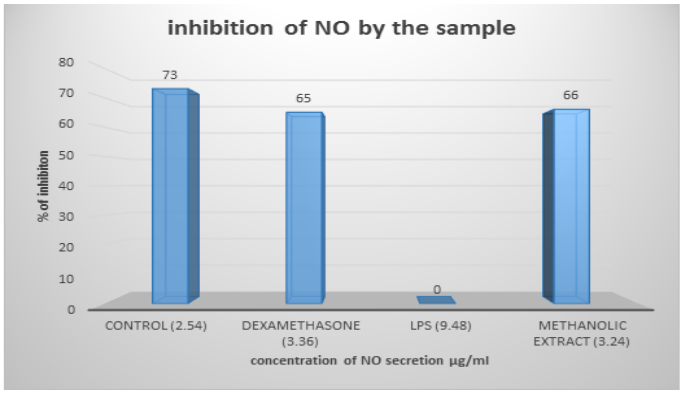

Fig 4: *In-Vitro* Anti-inflammatory by Nitric oxide method, data expressed as Percent inhibition of Nitric oxide of both the sample and Dexamethasone compared to the LPS- stimulated cells alone.

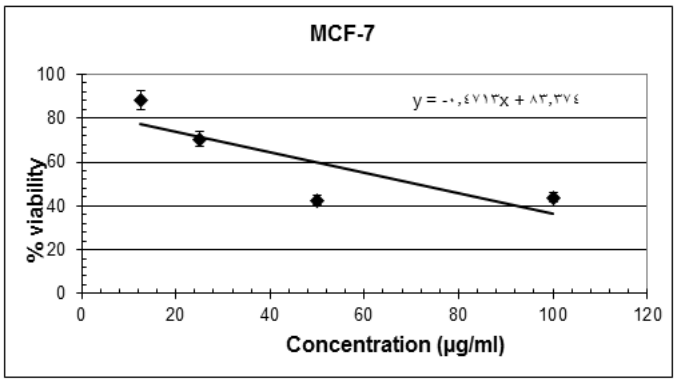

Fig 5: Cytotoxic effect of methanolic extract aginst MCF-7 cell using MTT assay (n=4), data expressed as the mean value of cell viability (% of control)= S.D

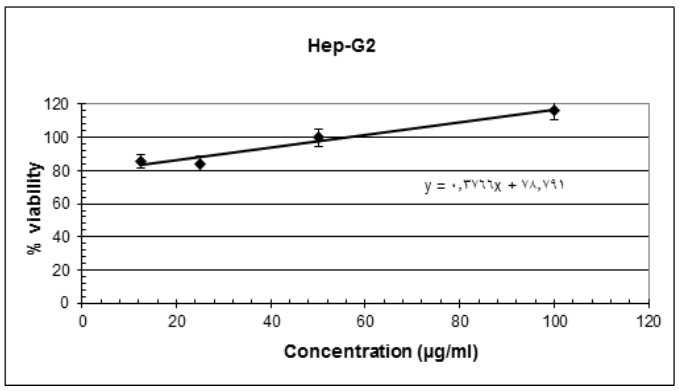

Fig 6: Cytotoxic effect of sample aginst Hep-G2 cell using MTT assay (n=4), dataex pressed as the mean value of cell viability (% of control)= S.D

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