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# Investigation of *Livistona decipiens* Leaf Methanol Extract and Evaluation of Antioxidant, Antimicrobial and Cytotoxic Activities

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## ABSTRACT

**Objectives:** This study aimed to isolate the polyphenolic constituents from the methanol extract of *Livistona decipiens* Becc leaves and evaluate the antioxidant, cytotoxic and antimicrobial activities of the total methanol extract and ethyl acetate fraction. **Methods:** The ethyl acetate and *n*-butanol fractions of *Livistona decipiens* leaf methanol extract were subjected separately to different chromatographic separation techniques. Structures of the isolated compounds were established by different spectroscopic techniques ($^{1}H$ / $^{13}C$ NMR). Antioxidant activity was evaluated by DPPH assay, while evaluation of cytotoxicity was done according to MTT cell viability assay. Antimicrobial activity was done by agar diffusion method. **Results:** seven compounds were isolated from the ethyl acetate and *n*-butanol fractions, five compounds were identified for the first time from this plant as apigenin-8-C-*β*-D-glucopyranoside (Vitexin) (**1**), Quercetin-6-C-*β*-D-glucopyranoside (**2**),apigenin-6,8-di-C-*β*-D-glucopyranoside (Vicenin II) (**3**), 6-*O*-methyl Kaempferol 3-*O*-glucopyranoside (**4**), luteolin-3-C-gentiobiosyl (**5**), while two compounds *vis;* luteolin-6-C-*β*-D-glucopyranoside (Isoorientin) (**6**) and luteolin-8-C-*β*-D-glucopyranoside (Orientin) (**7**), were isolated for the second time from this species. The ethyl acetate fraction has shown moderate activity against Gram positive and Gram negatvie bacteria, also it showed moderate antioxidant activity with $IC_{50}$ = 23 ± 0.74 *µ*g/ml when compared to ascorbic acid $IC_{50}$ = 14.2 ± 0.35 *µ*g/ml. Also ethyl acetate extract has shown cytotoxic activity on MCF-7 cells (human breast cancer cell line), HepG-2 (human hepatocellular carcinoma) and HeLa cells (human cervical cancer cell line), whereas, the methanol extract has shown lower activity. **Conclusion:** *Livistona decipiens* have potential medicinal value being rich in polyphenolics and being antioxidant, cytotoxic and antimicrobial drug.

***Keywords:*** *Antimicrobial, Antioxidant, Cytotoxicity, DPPH, Livistona decipiens, MTT, Polyphenolic*

## INTRODUCTION

Arecaceae, also called Palmae, is among the best known and extensively cultivated plant families[1]. The family has been neglected chemically, probably because of the difficulty of collecting fresh material and getting it authenticated. Most work has been carried out on economically important palms such as *Phoenix dactylifera*, *Cocos nucifera* and other palms cultivated for their oils[2]. Among its genera is *Livistona* R. Br. known as Fountain palm, which is a genus of 34 or possibly more species of hermaphrodite, shrubby or arborescent palms, comprising the most common fan-palms of greenhouses and decorative use, native to East Asia and Australia. *Livistona* are widely cultivated as ornamentals in the tropics and subtropics regions[3]. *L. decipiens* Becc leaves are up to 5 ft. long, very deeply divided into about 80 segments. Petioles often toothed only near the base; inflorescence, with glabrous bracts or with hairs only on the margins of smaller bracts, bearing flowers, sepals with solid base as long as erect narrow lobes; fruit Blackish, 1/2 inch in diameter[4]. Reviewing the current literature, it was found that little work was reported on genus *Livistona* which showed that plants belonging to this genus are rich in flavonoids, phenolic acids, ceramides, glycerides, lipids and acylglucosylsterols[1,5-8]. The few phytochemical reports on *L. decipiens* Becc showed that the lipoidal

matter of its fruit pulp has antihyperlipidimic and ulceroprotective activity[8]. That was encouraging to investigate this plant species phytochemically and biologically.

## MATERIALS AND METHODS

### Plant material

The leaves of *L. decipiens* were collected from Al-Orman garden, Giza, Egypt in 22 April 2014. The plant was identified by Dr. Terase Labib, Department of Flora and Taxonomy, Al-Orman Botanical Garden, Giza, Egypt, A voucher specimen No. 01Lde/2014 of *Livistona decipiens* Becc was kept in the herbarium in the Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

### Materials for biological studies

#### *Mammalian cell lines*

MCF-7 cells (human breast cancer cell line), HepG-2 (human hepatocellular carcinoma) and HeLa cells (human cervical cancer cell line), were obtained from the American Type Culture Collection (ATCC, Rockville, MD), **Fungi:** *Aspergillus fumigatus* (RCMB 002008) and *Candida albicans* RCMB 005003 (1) ATCC 10231, **Gram positive bacteria:** *Staphylococcus aureus* (RCMB010010), *Bacillus subtilis* RCMB 015 (1) NRRL B- 543, **Gram negative bacteria:** *Salmonella typhimurium* RCMB 006 (1) ATCC 14028, *Escherichia coli* (RCMB 010052), ATCC 25955.

#### *Reagents for biological studies*

Dimethyl sulfoxide (DMSO), MTT and trypan blue dye was purchased from (Sigma-Aldrich) (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, PBS buffer solution, L-glutamine and 0.25% Trypsin-EDTA were purchased from Lonza (Belgium) and 50µg/ml gentamycin was purchased from (Sigma-Aldrich) with 5% $CO_2$ were used for evaluation of cytotoxic activities. Freshly prepared (0.004%w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10˚C in the dark were used for evaluation of antioxidant activities.

### Standard material

*Authentic flavonoids and sugars*

Authentic flavonoids; including Luteolin, Apigenin, Quercetin, Kaempferol, as well as standard sugars; including glucose, rhamnose, xylose, galactose, gentiobiose, were all available in the Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

#### *Reference drugs for biological study*

Ascorbic acid, Ketoconazole, Gentamycin and Vinblastine Sulfate were supplied by RCMB: Regional Center for Mycology and Biotechnology, Cairo, Egypt.

### Materials for chromatographic techniques

Silica gel G60 for column chromatography (70-230 mesh, Merk), Silica gel G60 for thin layer chromatography (E. Merk, Germany), Silica gel GF254 for pre-coated TLC plates (E. Merk, Germany), Sephadex LH-20 (Sigma-Aldrich-Steinheim, Germany), Sheets of Whatman filter paper (1mm) for paper Chromatography (Whatman Itd, Maid stone, Kent, England), micro crystalline cellulose (E. Merk-Darmstadt, Germany) and polyamide 6S (Riedel-De-Haen AG, SeelzeHaen AG, SeelzeHanver, Germany). Paper chromatography (Whatman No. 1) was performed using solvent systems: (1) BAW (n-BuOH – HOAc – $H_2O$ 4: 1: 5, upper layer), (2) 15% HOAc ($H_2O$ – HO Ac 85:15)[9].

### Apparatus and equipment for chromatographic techniques

Rotary evaporator (Buchi, A.G. Switzerland), glass column for chromatography (Ø 120 x 3.5 cm), Buchner filter funnel, analytical balance (Melter H20, USA), micropipettes for spotting, rectangular glass tank 50 cm x 56 cm x 20 cm was used for PC and 24 cm x 24 cm x 10 cm for TLC, Ultraviolet lamp for localization of spots on paper and thin layer chromatography, (NMR) Nuclear magnetic resonance spectrometer, Bruker a 400, MHz for $^1$H NMR and 100.40 MHz for $^{13}$C NMR. The spectra were run in DMSO, and chemical shifts were given in ppm with tetramethylsilane (TMS) as an internal standard.

### Apparatus for biological studies

A microplate reader (SunRise, TECAN, Inc, USA), the 96 - well plate used for cytotoxicity evaluation using cell viability assay, a UV-visible spectrophotometer (Milton Roy, Spectronic 1201), used for measuring the absorbance in the antioxidant assay.

### Methods

### Extraction and isolation of phenolic compounds

The air dried leaves of *L. decipiens* (2 kg) were coarsely ground and extracted three times with 10 L 100% methanol. Then, the leaf extracts were combined and evaporated to dryness under reduced pressure to yield 306 g concentrate. The dried leaf concentrate was reconstituted with 300 ml $H_2O$ and then was fractionated with 3 × 300 ml of petroleum ether, ethyl acetate and *n*-butanol respectively, by liquid-liquid phase separation yielding four fractions weighing (160 g petroleum ether fraction, 10 g ethyl acetate fraction,

20 g *n*-butanol fraction and 116 g aqueous fraction). 2D-PCof the fractions revealed the presence of a pronounced number of flavonoid spots in the ethyl acetate and *n*-butanol fractions, which were detected under UV-light and with specific spray reagents. Thus both ethyl acetate and n-butanol fractions were selected for further phytochemical investigation.

**The ethyl acetate fraction** (10 g) was fractionated on polyamide column (Ø 4 × 70 cm), the column was eluted using water then $H_2O$ / MeOH mixtures with decreasing polarity to yield 30 individual fractions (150 ml) each, collected into 5 main fractions based on their similarity on PC (**I-V**), the promising fraction (based on spots detected under UV) was **FIII** (F24-58), (0.7 g), which was re chromatographed on another polyamide column using 20% MeOH / $H_2O$, giving four subfractions were afford (**FIII** a – **FIII** e). **FIII c** (0.1 g) showed two dark purple spots with minor yellow spot ( under UV on PC ($S_1$) ) then applied on Sephadex LH-20 column for separation using mobile phase *n*-butanol saturated with water affording compound **1** (11 mg) and compound **2** (20 mg).

**The*n*-butanol fraction** (20 g) was fractionated on polyamides column (Ø 5 × 90 cm), the column was eluted using water then $H_2O$ / MeOH mixtures with decreasing polarity from 100% $H_2O$ to 0% $H_2O$ to yield 50 individual fractions, collected into 6 main fractions (**FI - FVI**) based on their similarity on PC, the most promising fractions were **FI** and **FII**. Fraction **FI** was re-chromatographed on Sephadex column using 100% MeOH to afford 5 subfractions (**FI** a- **FI** e). **FI** b (0.3 g) was re-chromatographed on Sephadex column using 20% $H_2O$/ MeOH to give compound **3** (10 mg).Fraction **FII** was re chromatographed on silica gel column using $CH_2CL_2$/MeOH mixture with increasing polarity (from 0% - 50% MeOH), to afford 7 subfractions (**FII** a – **FII** g). Several chromatographic separations on cellulose columns using mobile phase *n*-butanol saturated with water led to the isolation of four compounds compound **4** (10 mg), compound **5** (20 mg), compound **6** (17 mg) and compound **7** (26 mg).

**Hydrolysis of glycosides**

Oxidative hydrolysis using ferric chloride (0.2 g in 0.8 ml $H_2O$) refluxed for 6 hours was performed for *C*-glycosides, while complete acid hydrolysis using 1.5 N HCl in aqueous methanol (50%), refluxed for 2 hours at 100ºC was performed for *O*-glycosides. Each hydroylsate was then extracted with ethyl acetate. The ethyl acetate extract was subjected to CoPC (comparative paper chromatography) investigation against authentic aglycone. The aqueous layer was then neutralized with sodium carbonate and subjected to CoPC against authentic sugars for identification of the sugar part[9].

**Antioxidant activity**

The antioxidant activity of extracts was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University by the DPPH free radical scavenging assay in triplicate and mean values were considered[10].

**DPPH radical scavenging activity:**

Freshly prepared (0.004 %w/v) methanol solution of 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical was prepared and stored at 10˚C in the dark. A methanol solution of the tested extracts were prepared with sample concentrations (0, 10, 20, 40, 80, 160, 320, 640, 1280 $\mu$g/mL). A 40 uL aliquot of the methanol solution was added to 3 ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer. The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage of DPPH radical scavenging was calculated according to the formula: (scavenging activity) = [{(*A*C- *A*T)/ *A*C} × 100] (1). Where *A*C = Absorbance of the control at t = 0 min and *A*T = absorbance of the sample + DPPH at t = 16 min[10].

**Antimicrobial activity**

The total methanol extract and ethyl acetate fraction of *L. decipiens* leaves were assayed for anti-microbial activity using the susceptibility tests. Screening tests regarding the inhibition zone were carried out by the well diffusion method[11]. The inoculums suspension was prepared from colonies grown overnight on an agar plate and inoculated into Mueller-Hinton broth (fungi using malt broth). A sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates (fungi using malt agar plates). The extracts were dissolved in dimethyl sulfoxide (DMSO) with different concentrations (10, 5, 2.5 mg/ml). The inhibition zone was measured around each well after 24 h at 37°C. Controls using DMSO were adequately done.

**Evaluation of cytotoxicity**

The total methanol extract and ethyl acetate fraction of *L. decipiens* leaves were evaluated against (HepG2), (MCF-7) and HeLa cell lines using the MTT cell viability assay.

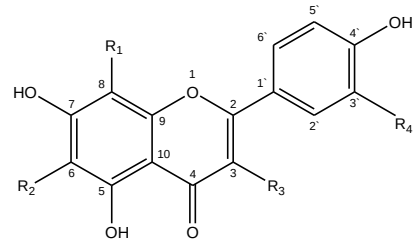
**Cell line propagation**

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and

**Table 1. $^{1}H$ NMR and $^{13}C$ NMR data of compounds 6 and 7**

| Compound 7 | | Compound 6 | | C-No |
|---|---|---|---|---|
| Hδ | δCδ | δh | δ C | |
| | 164.57 | | 164.17 | 2 |
| 6.65,s | 102.75 | 6.68, s | 103.11 | 3 |
| | 182.54 | | 182.40 | 4 |
| | 160.99 | | 163.73 | 5 |
| 6.28,s | 98.48 | | 109.19 | 6 |
| | 162.98 | | 160.97 | 7 |
| | 104.91 | 6.49, s | 93.91 | 8 |
| | 156.33 | | 156.68 | 9 |
| | 104.31 | | 104.09 | 10 |
| | 122.33 | | 121.75 | 1' |
| 7.49,d,(2.4) | 114.48 | 7.41 ,d, (2.4) | 113.91 | 2' |
| | 146.51 | | 146.14 | 3' |
| | 150.04 | | 150.07 | 4' |
| 6.86,d,(8.97) | 116.06 | 6.90, d, (8.25) | 116.54 | 5' |
| 7.54,dd,(2.4 & 8.97) | 119.97 | 7.44,dd, (2.4 & 8.25) | 119.23 | 6' |
| 4.69,d,(9.83) | 73.48 | 4.59,d,(9.81) | 73.50 | 1'' |
| 4.99,s | 71.17 | 4.05,t-like | 71.14 | 2'' |
| 3.7-.89,m,remaining of sugar protons | 79.03 | 3.75-3.89,remaning of sugar protons | 79.18 | 3'' |
| | 62.10 | | 70.41 | 4'' |
| | 82.37 | | 81.92 | 5'' |
| | 56.87 | | 61.94 | 6'' |

*$^{1}H$ NMR (400 MHz, DMSO-d6), $^{13}C$ NMR (100.40 MHz, DMSO-d6), values between parentheses represent the J-values in Hz*




| | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Compound 1 | Glu | H | H | H |
| Compound 2 | H | Glu | OH | OH |
| Compound 3 | Glu | Glu | H | H |
| Compound 4 | H | H | Gentiobiosyl | OH |
| Compound 5 | H | $OCH_3$ | O- Glu | H |
| Compound 6 | H | Glu | H | OH |
| Compound 7 | Glu | H | H | OH |

**Figure 1. Chemical structures of the isolated compounds from the leaves of *Livistona decipiens***

50 µg/ml gentamycin + ampicillin. The cells were maintained at 37ºC in a humidified atmosphere with 5% $CO_2$ and were subcultured two to three times a week.

**Cytotoxicity evaluation using viability assay**

For cytotoxicity assays, the cancer cell lines were suspended in medium at concentration 5 × $10^4$ cell/well in Corning® 96 - well tissue culture plates, and then incubated for 24 hr. The tested methanol and ethyl acetate extracts were then added into 96 - well plates (three replicates) to achieve twelve concentrations for each extract. Six vehicle controls with media or 0.5 % DMSO were run for each 96 - well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 - well plates and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 - well plates were then incubated at 37°C and 5% $CO_2$ for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the micro plate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)] × 100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug plotted to get the survival curve of each tumor cell line. The 50% inhibitory concentration ($IC_{50}$), required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration. using Graph pad Prism software (San Diego, CA. USA)[12,13].

**Statistical Analysis**

All experimental results were expressed as means ± SD. Analysis of variance was performed by ANOVA procedures. Correlation coefficient ($R^2$) was used to determine two variables. SPSS software was used for statistical calculations. The results with $P<0.05$ were regarded to be statistically significant.

## RESULTS AND DISCUSSION

**Characterization and identification of isolated compounds**

Chromatographic separation of ethyl acetate and *n*-butanol fractions of *L. decipiens* leaves resulted in seven compounds. Structures of the isolated compounds (Figure 1) were identified by different spectral techniques including $^1$H NMR, $^{13}$C NMR, and also by CoPC against standard authentic sugars and aglycones after complete acid hydrolysis.

**Compound 1:** Isolated as yellow amorphous powder (11 mg), chromatographic properties; $R_f$-values (0.43 $S_1$, 0.49 $S_2$) and it gave dark purple spot under UV-light, turned to yellowish green on exposure to $NH_3$ vapors, grayish yellow fluorescence on exposure to Naturstoff spray reagent and green color with $FeCl_3$, $^1$H NMR (400 MHz, DMSO-d6), δ ppm 8.02 (2H, d, *J* = 7.49 Hz, H-2′/6′), 6.91 (2H, d, *J* = 8.28 Hz, H-3′/5′), 6.77 (1H, s, H-3), 6.29 (1H, s, H-6), 4.71 (1H, d, *J* = 9.23 Hz, H-1″), 3.2-3.9 (m, remaining of sugar protons). $^{13}$C NMR (100 MHz, DMSO-d6), δ ppm 182.49 (C-4), 167.48 (C-2), 164.36 (C-7), 161.58 (C-4′), 156.33 (C-9), 160.33 (C-5), 129.39 (C-2′/6′), 122.17 (C-1′), 116.50 (C-3′/5′), 109.78 (C-8), 105.30 (C- 10), 102.71 (C-3), 98.88 (C-6), 82.10 (C-5″), 79.14 (C-3″), 73.88 (C-1″), 71.34 (C-2″), 71.00 (C-4″), 61.55 (C-6″).

Based on its chromatographic properties it was expected to be an apigenin structure[9]. This expectation was supported by$^1$H NMR spectrum which showed an $A_2X_2$ spin coupling system of two ortho-doublets, each integrated for two protons at δ ppm 8.02 (*J* = 7.49 Hz H-2′/6′) and 6.91 (*J* = 8.28 Hz H-3′/5′) indicated 4′-hydroxy B-ring. The glycoside moiety in compound **1** was identified as *β*-C- glycoside from doublets at δ 4.71 with large *J* value 9 Hz). Absence of H-8 signal from $^1$H NMR spectrum of compound **1** has led to conclude that the C-glycosidation must be on C-8. This evidence was confirmed from downfield shift of C-8 to 109.78 (~ + 10 ppm) in $^{13}$C NMR spectra of compound 1, Moreover, the C-glycoside moiety in the structures was confirmed as *β*-C-glucopyranoside depending on the characteristic up field location of C-1″ at δ 73.88 ppm and downfield location of both C-5″ and C-3″ at δ 82.10 and 79.14 ppm, respectively, with respect to those of *O*-glycosides. The assignment of all other $^{13}$C resonances of compound **1** was achieved by comparison with the corresponding data of structural related compounds[9,14-18,29].According to the above discussed data compound **1** was confirmed as apigenin-8-C-*β*-D-glucopyranoside (Vitexin) which was isolated for the first time from *L. decipiens.*

**Compound 2**: Isolated as a deep yellow color crystal with $R_f$ value (0.82 $S_1$), $^1$H NMR: δ ppm 7.7 (1H, d, *J* = 2.40 Hz, H-2′), 7.41(1H, dd, *J* = 2.40 & 8.28Hz, H-6′), 6.79 (1H, d, *J* = 8.28 Hz, H-5′), 6.48 (1H, s, H-8), 4.73 (1H, d, *J* = 9.75Hz, H-1″), 3.2-3.9 (m, remaining of sugar protons),$^{13}$C NMR (100 MHz, DMSO-d6), δ ppm 176.28 (C-4), 163.80 (C-7), 160.90 (C-5), 156.69 (C-9), 151.85 (C-4′) 147.06 (C-3′), 146.80 (C-2), 138.56 (C-3), 125.09 (C-1'), 119.70 (C-6′),116.25 (C-5′), 113.58(C-2′), 109.71 (C-6),

**Table 2. Antioxidant activity (scavenging activity) of methanol extract and ethyl acetate fraction of *Livistonadecipiens* leaves compared to ascorbic acid.**

| Sample conc. (µg/ml) | Mean % of DPPH (scavenging activity) | | | | | |
|---|---|---|---|---|---|---|
| | Total methanolextract of*L.decipiens* | | Ethyl acetate fraction of *L. decipiens* | | Ascorbic acid | |
| | Mean | S.D. (±) | Mean | S.D. (±) | Mean | S.D. (±) |
| 1280 | 81.75 | 1.30 | 87.39 | 1.58 | 93.45 | 1.08 |
| 640 | 78.58 | 0.48 | 83.58 | 0.72 | 88.64 | 0.99 |
| 320 | 76.55 | 1.36 | 82.24 | 0.64 | 80.92 | 1.64 |
| 160 | 73.74 | 0.74 | 79.83 | 0.69 | 75.68 | 1.59 |
| 80 | 69.44 | 3.71 | 77.49 | 0.86 | 66.13 | 4.45 |
| 40 | 38.02 | 1.14 | 72.21 | 1.32 | 53.21 | 1.81 |
| 20 | 22.12 | 2.38 | 46.07 | 1.17 | 20.31 | 3.11 |
| 10 | 17.03 | 1.51 | 32.39 | 1.43 | 11.80 | 2.09 |
| 0 | 100 | --- | 100 | ---- | 100 | ----- |

*Results are means ± SD (n=**3**) P<0.05*

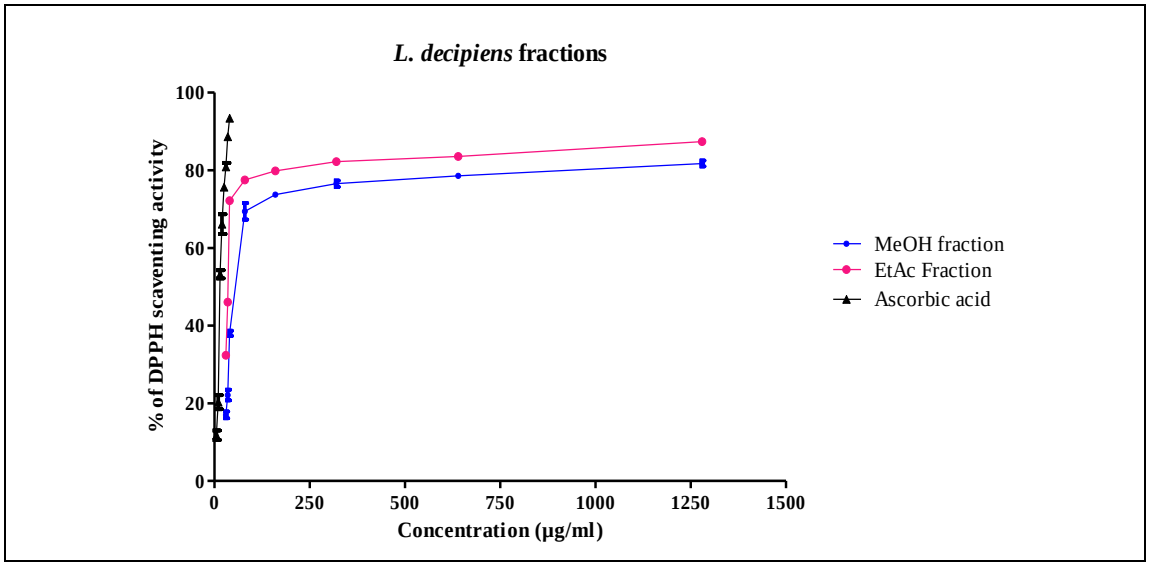



**Figure 2. Antioxidant activity (scavenging activity) of methanol extract and ethyl acetate fraction of *Livistona decipiens* leaves compared to ascorbic acid**

104.28 (C-10), 93.60 (C-8), 81.27 (C-5″), 79.42 (C-3″), 73.66 (C-1″), 71.40 (C-2″), 70.57 (C-4″), 61.37 (C-6″).

$^{1}$H NMR spectra of compound **2** showed ABX spin coupling system of δ ppm 7.7, 7.41, 6.79 for H-2′, H-6′, H-5′ respectively, characteristic for 3′,4′ dihydroxy B-ring, the presence of H-8 as singlet signal together with doublet signal with large *J*-value at δ ppm 4.73 (1H, d, *J* = 9.75)H-1″, corresponding to the sugar moiety which indicate 6-C-glycosylation.$^{13}$C NMR spectrum of **2**, showed well resolved typical 15 signals of quercetin aglycone moiety, the down field shifts of C-6 to 109.71 (~+ 10 ppm) was confirmative evidence for the C-glycosylation at C-6. Moreover the-C-glycoside moiety was confirmed as 6-C-*β*-glucopyranoside depending on the intrinsic up field location of C-1″ (anomeric carbons) at δ ppm 73.66 and downfield location of C-5″, C-3″ at δ ppm 81.27, 79.42, with respect to those of *O*-glycosides. The assignment of all other $^{13}$C resonances of the compound was achieved by comparison with the corresponding data of structural related compounds[9,19-21]. According to the above discussed data compound **2** was confirmed as

quercetin-6-C-*β*-D-glucopyranoside, which was isolated for the first time from genus *Livistona*.

**Compound 3:** Isolated as yellowish amorphous powder (10 mg), chromatographic properties: $R_f$ values: 0.38 ($S_1$), 0.72 ($S_2$); it showed deep purple color under UV-light turned to yellow-green on exposure to $NH_3$ vapor and gave grayish yellow fluorescence and green color with Naturstoff and $FeC1_3$ spray reagents, respectively.$^1$H NMR (400 MHz, DMSO-d6), δ ppm 8.03 (2H, d, *J* = 8.39 Hz, H-2′/6′), 6.92 (2H, d, *J* = 8.60 Hz, H-3′/5′), 6.82 (1H, s, H-3), 4.81 (1H, d, *J* = 9.94 Hz, H-1″), 4.77 (1H, d, *J* = 10 Hz, H-1‴), 3.3-3.8 (m, 12 sugar protons). $^{13}$C NMR (100 MHz, DMSO-d6), δ ppm 180.36 (C-4), 164.61 (C-2), 162.45 (C-7), 161.76 (C-4′), 158.48 (C-9), 155.55 (C-5), 129.51 (C-2′/6′), 121.90 (C-1′), 116.34 (C-3′/5′), 107.96 (C-6), 105.44 (C-8), 103.02 (C- 10), 100.92 (C-3), 82.61 (C-5″), 82.32 (C-5‴), 78.95 (C-3″), 77.91 (C-3‴), 74.21 (C-1″), 73.69 (C-1‴), 71.02 (C-2″), 70.98 (C-2″), 70.23 (C-4″), 69.48 (C-4‴), 61.65 (C-6″), 60.62 (C-6‴).

According to the chromatographic properties, compound **3** was expected to be apigenin structure[9]. $^1$H NMR spectrum a flavone compound was confirmed by the appearance of a singlet at δ 6.81 for H-3. Additionally, the spectrum showed $A_2X_2$ spin coupling system of two ortho-doublets each integrated for two protons, at δ ppm 8.03 (2H-2′/6′) and 6.92 (2H-3′/5′) for 4′-hydroxy B-ring expecting an apigenin nucleus. The absence of H-6 and H-8 signals from the spectrum and the presence of two doublets of large *J* value 10 Hz and 9.94 Hz at δ ppm 4.81 and 4.77 respectively, were attributed to two anomeric protons of two C-*β*-D-glucopyranoside moieties. This was indicative to 6,8-di-C-*β*-glycosyl apigenin structure.$^{13}$C NMR spectrum of Compound **3** showed well resolved typical fifteen signals of an apigenin aglycone moiety, including the four key signals for C-4′, C-2′/6′, C-3′/5′ and C-3 at δ ppm 161.76, 129.51, 116.34 and 100.92, respectively. The down field shifts of $^{13}$C-resonance of C-6 to 107.96 and C-8 to 105.44 (~ + 10 ppm) was confirmative evidence for the C-glycosylation at C-6 and C-8. Moreover the di-C-glycoside moiety was confirmed as 6,8-di-C-*β*-glucopyranoside depending on the intrinsic up field location of C-1″ and C-1‴ (anomeric carbons) at δ ppm 74.21 and 73.69, respectively and downfield location of C-5″, C-5‴, C-3″ and C-3‴ at δ ppm 82.61, 82.32, 78.95 and 77.91, respectively, with respect to those of *O*-glycoside. The assignment of all other $^{13}$C resonances of compound **3** was achieved by comparison with the corresponding data of structural related compounds[14-17, 29]. According to the above discussed data compound **3** was confirmed as apigenin-6,8-di-C-*β*-D-glucopyranoside (Vicenin II), which was isolated for the first time from genus *Livistona*.

**Compound 4:** Isolated as yellowish amorphous powder (10 mg), chromatographic properties: $R_f$ values: 0.77 ($S_1$), 0.08 ($S_2$); it showed deep purple color under UV-light turned to yellow on exposure to $NH_3$ vapor and gave orange fluorescence and green color with Naturstoff and $FeC1_3$ spray reagents respectively, $^1$H NMR (400 MHz, DMSO-d6),δ ppm 7.68 (1H, dd, *J* = 1.97 & 8.44 Hz, H-6′), 7.54 (1H, d, *J* = 1.97, H-2′), 6.82 (1H, d, *J* = 8.44 Hz, H-5′), 6.41 (1H, d, *J* = 1.56Hz, H-8), 6.21 (1H, d, *J* = 1.56Hz, H-6), 5.38 (1H, d, *J* = 7.66Hz, H-1‴),. 4.60 (1H, d, *J* = 9.83Hz, H-1″)$^{13}$C NMR (100 MHz, DMSO-d6), δ ppm, 182.43 (C-4), 164.64 (C-2), 161.69 (C-5/7), 156.70 (C-9), (148.94 (C-4′), 122.42 (C-1′), 121.62 (C-6′), 116.65 (C-5′), 109.32 (C-3), 104.58 (C-1‴), 102.27 (C-10), 99.14 (C-6), 93.99 (C-8), 82.01 (C-5″), 79.58 (C-2‴), 79.39 (C-3″), 76.95 (C-3‴), 76.29 (C-5‴), 73.60 (C-4‴), 73.51 (C-1″), 71.66 ( C-2″), 71.06 (C-4″), 70.37 (C-6″), 63.01(6‴).

Compound **4** was expected to be a luteolin structure[9]. $^1$H NMR spectra showed an ABX-spin coupling system of three proton resonances at δ ppm,7.58 (1H, dd, *J* = 1.97, 8.44 Hz, H-6′), 7.53 (1H, d, *J* = 1.97, H-2′) and ortho-doublet at 6.83 (1H, d, *J* = 8.44 Hz, H-5′) for compound **4** to indicate a 3′,4′ dihydroxy B-ring., additionally the presence of two meta coupled doublets (each integrated to one proton) of two protons at δ 6.41 and 6.21 (ppm) of H-8 and H-6 respectively, indicating 5,7-dihydroxy A-ring. Absence of H-3 signal from $^1$H NMR of compound **4** has led to conclude that the C-glycosylation must be on C-3 and the presence of doublet at δ 4.60 with large *J* values (> 9 Hz) for the anomeric proton of sugar moiety1 H-1″ of compound **4** were intrinsic for a *β*-C-glycoside moiety. As further confirmation, $^{13}$C NMR spectrum for compound **4** showed well-resolved typical 15 signals of a luteolin aglycon moiety, Additionally, the C-glycoside moiety in structures was confirmed as *β*-C-glucopyranoside depending on the characteristic up-field location of C-1″ at 73.50 ppm, and downfield location of both C-5″ and C-3″ to δ 82.01 and 79.39 ppm for the internal sugar moietywith respect to those of *O*-glycosides[19]. The C-glycosylation at C-3 in compound **4** was concluded from the downfield shift of C-3 to δ 109.32 (~ + 10 ppm). Additionally, the *O*-glycoside linkage in structures was confirmed, depending on the characteristic down-field location of C-6" at δ 70.37 ppm. Also the presence of doublet at δ 5.39 for the anomeric proton H-1‴ of the terminal sugar moiety was intrinsic for (1‴ - 6″) *O*-glycoside moiety [28]. The assignment of all other $^{13}$C NMR resonances of compound **4** was achieved by comparison with the corresponding data of structural related compounds [9,15,19,22,23]. According to the above discussed data, compound **4** was confirmed as luteolin-3-C-*β*-D-

**Table 3. Antimicrobial activity of methanol extract and ethyl acetate fraction of *Livistona decipiens* leaves**

| Sample | Total methanolextract of *L. decipiens* | | Ethyl acetate fraction of *L. decipiens* | | Control | |
|---|---|---|---|---|---|---|
| Tested microorganisms | Mean IZ | S.D. (±) | Mean IZ | S.D. (±) | Mean IZ | S.D. (±) |
| Fungi | | | | | Ketoconazole | |
| ***Aspergillus fumigatus*** | NA | NA | NA | NA | 21.01 | 0.02 |
| ***Candida albicans*** | NA | NA | NA | NA | 23.03 | 0.04 |
| Gram Positive Bacteria | | | | | Gentamycin | |
| ***Staphylococcus aureus*** | 10.50 | 0.50 | NA | NA | 30.01 | 0.01 |
| *Bacillus subtilis* | 12.50 | 0.50 | 10.50 | 0.50 | 26.02 | 0.03 |
| Gram Negatvie Bacteria | | | | | Gentamycin | |
| ***Salmonella typhimurium*** | NA | NA | 9.50 | 0.50 | 33.02 | 0.03 |
| ***Escherichia coli*** | NA | NA | 7.83 | 0.76 | 27.09 | 0.01 |

**NA: No activity. The sample was tested at concentration.10 mg/ml.*

**Table 4. Cytotoxic activity (viability %) of methanol extract and ethyl acetate fraction of *Livistona decipiens* leaves on MCF-7cell line compared to Vinblastine Sulfate**

| Sample conc. (µg/ml) | Mean of Viability% MCF-7 cell line | | | | | |
|---|---|---|---|---|---|---|
| | Total methanol extract of *L. decipiens* | | Ethyl acetate fraction of *L. decipiens* | | Vinblastine Sulfate | |
| | Mean | S.D. (±) | Mean | S.D. (±) | Mean | S.D. (±) |
| 500 | 36.18 | 1.50 | 16.32 | 0.86 | 4.12 | 0.17 |
| 250 | 47.92 | 1.49 | 31.96 | 2.59 | 7.24 | 0.38 |
| 125 | 70.83 | 2.30 | 42.00 | 4.30 | 13.78 | 0.87 |
| 62.5 | 88.68 | 2.16 | 57.14 | 4.92 | 19.00 | 2.22 |
| 31.25 | 97.05 | 1.87 | 73.41 | 3.15 | 26.40 | 3.01 |
| 15.6 | 99.59 | 0.72 | 88.80 | 2.26 | 37.05 | 1.47 |
| 7.8 | 100.00 | 0.00 | 97.93 | 2.23 | 43.96 | 2.28 |
| 3.9 | 100.00 | 0.00 | 99.88 | 0.20 | 53.96 | 2.06 |
| 2.00 | 100 | --- | 100 | --- | 61.38 | 2.52 |
| 1.00 | --- | --- | ---- | --- | 69.21 | 3.73 |
| 0 | --- | --- | ---- | ----- | 100 | ----- |

*Results are means ± SD (n=3) P<0.05*

glucosyl (1‴ - 6″) glucopyranoside (luteolin-3-C-gentiobioside), was isolated for the first time from *L. decipiens.*

**Compound 5:** Obtained asyellowish amorphous powder (20 mg), Chromatographic properties: $R_f$ values: 0.47 ($S_1$), 0.36 ($S_2$); it showed deep purple color under UV-light turned to yellow on exposure to $NH_3$ vapor and gave yellowish-green fluorescence and pale green color with Naturstoff and $FeC1_3$ spray reagents respectively, $^1$H NMR (400 MHz, DMSO-d6), 7.67 (2H, d, J = 8.47 Hz, H-2′/6′), 6.94 (2H, d, *J* = 8.69Hz, H-3′/5′), 6.52 (1H, s, H-8), 5.38 (1H, d, *J* = 7.66Hz, H-1″), 3.82 (3H, s, $OCH_3$ at C-6), 3.2-3.7 (m, remaining of sugar protons). $^{13}$C NMR (100 MHz, DMSO-d6), δ ppm, 177.94 (C-4), 164.62 (C-7/5), 161.69 (C-4′), 156.79 (C-2/9), 133.93 (C-3), 133.77, (C-3′, 5′), 128.94 (C-6), 122.49 (C-1′), 116.42 (C-2′, 6′), 104.37 (C-10), 102.27 (C-1″), 93.99 (C-8), 79.39

**Table 5. Cytotoxic activity (viability %) of methanol extract and ethyl acetate fraction of *Livistona decipiens* leaves on HepG-2 cell line compared to Vinblastine Sulfate**

| Sample conc. (µg/ml) | Mean of Viability% HepG-2 cell line | | | | | |
|---|---|---|---|---|---|---|
| | Total methanol extract of *L. decipiens* | | Ethyl acetate fraction of *L. decipiens* | | Vinblastine Sulfate | |
| | Mean | S.D. (±) | Mean | S.D. (±) | Mean | S.D. (±) |
| 500 | 34.61 | 2.87 | 14.19 | 2.22 | 3.91 | 0.16 |
| 250 | 48.03 | 2.66 | 25.18 | 1.67 | 6.77 | 0.35 |
| 125 | 75.13 | 1.83 | 37.71 | 2.55 | 10.38 | 0.44 |
| 62.5 | 90.69 | 1.16 | 53.31 | 4.06 | 14.89 | 1.68 |
| 31.25 | 98.17 | 0.81 | 73.57 | 2.96 | 24.33 | 2.33 |
| 15.6 | 100.00 | 0.00 | 90.35 | 1.86 | 33.00 | 1.09 |
| 7.8 | 100.00 | 0.00 | 98.31 | 1.14 | 41.94 | 1.24 |
| 3.9 | 100.00 | 0.00 | 100.00 | 0.00 | 47.62 | 1.83 |
| 2.00 | 100 | --- | 100 | --- | 56.84 | 2.48 |
| 1.00 | --- | --- | ---- | --- | 65.76 | 1.19 |
| 0 | --- | --- | ----- | --- | 100 | --- |

*Results are means ± SD (n=**3**) P<0.05*

**Table 6. Cytotoxic activity (viability %) of methanol extract and ethyl acetate fraction of *Livistonadecipiens* leaves on HeLa cell line compared to Vinblastine Sulfate**

| Sample conc. (µg/ml) | Mean of Viability% HeLa cell line | | | | | |
|---|---|---|---|---|---|---|
| | Total methanol extract of *L. decipiens* | | Ethyl acetate fraction of *L. decipiens* | | Vinblastine Sulfate | |
| | Mean | S.D. (±) | Mean | S.D. (±) | Mean | S.D. (±) |
| 500 | 40.54 | 2.79 | 20.48 | 0.98 | 5.12 | 0.89 |
| 250 | 64.06 | 3.99 | 33.68 | 1.79 | 9.08 | 1.33 |
| *125* | 82.11 | 3.69 | 47.40 | 2.10 | 15.31 | 1.29 |
| 62.5 | 94.82 | 2.79 | 71.11 | 3.22 | 23.79 | 2.79 |
| 31.25 | 99.37 | 0.66 | 86.20 | 2.64 | 29.96 | 3.16 |
| 15.6 | 100.00 | 0.00 | 96.61 | 1.11 | 38.47 | 2.57 |
| 7.8 | 100.00 | 0.00 | 99.41 | 0.79 | 46.42 | 0.70 |
| 3.9 | 100.00 | 0.00 | 100.00 | 0.00 | 57.55 | 3.05 |
| 2.00 | 100 | --- | 100 | --- | 66.50 | 2.35 |
| 1.00 | --- | ---- | ---- | ---- | 73.66 | 2.37 |
| 0 | ---- | ----- | ----- | ----- | 100 | ----- |

*Results are means ± SD (n=**3**) P<0.05*

**Table 7. $IC_{50}$ values of the standard drug, methanol extract and ethyl acetate fraction of *L. decipiens* leaves**

| Tested sample | MCF-7 | HeLa | HepG2 |
|---|---|---|---|
| **Methanol extract of *L. decipiens*** | $IC_{50}$ =238.6 ± 7.13 µg/ml | $IC_{50}$ =399 ± 24.5 µg/ml. | $IC_{50}$ =240.9 ± 9.07 µg/ml. |
| **Ethyl acetate fraction of *L. decipiens*** | $IC_{50}$ =91.9 ± 15.2 µg/ml. | $IC_{50}$ =118 ± 4.5 µg/ml. | $IC_{50}$ =75.7 ± 11.86 µg/ml. |
| **Vinblastine Sulfate** | $IC_{50}$ =5.44± 0.57 µg/ml | $IC_{50}$ = 6.54± 0.39 µg/ml. | $IC_{50}$ = 3.48 ± 0.22 µg/ml. |

*Results are means ± SD (n=**3**) P<0.05*

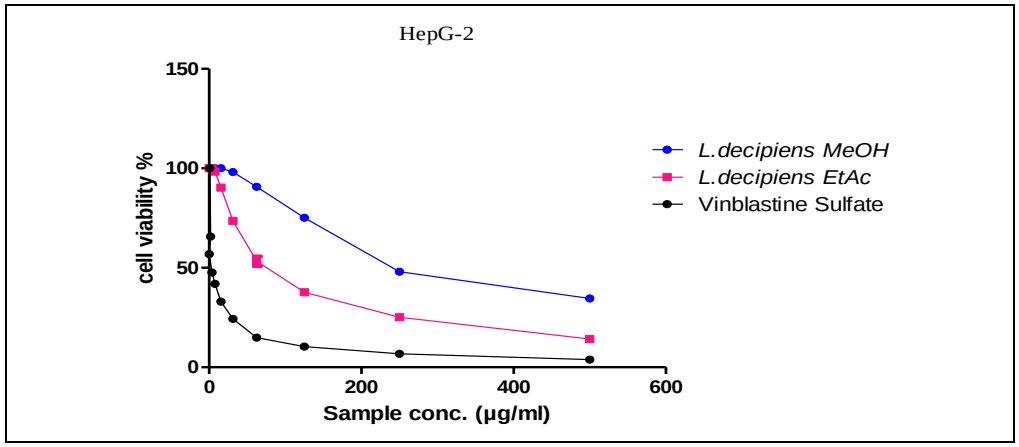



**Figure 3. Cytotoxic activity (viability %) of methanol extract and ethyl acetate fraction of *Livistona decipiens* leaves onHepG2 cell line compared to Vinblastine Sulfate**

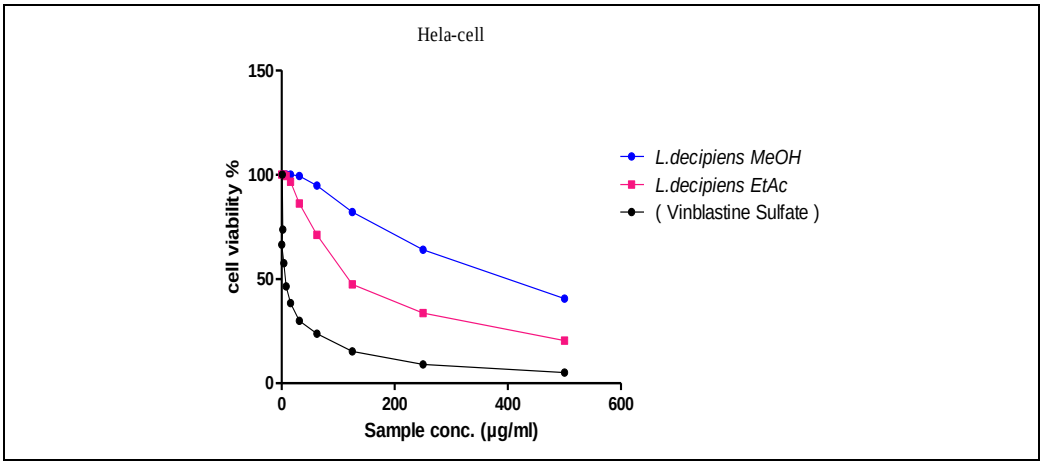



**Figure 4. Cytotoxic activity (viability %) of methanol extract and ethyl acetate fraction of *Livistona decipiens* leaves on HeLa cell line compared to Vinblastine Sulphate**

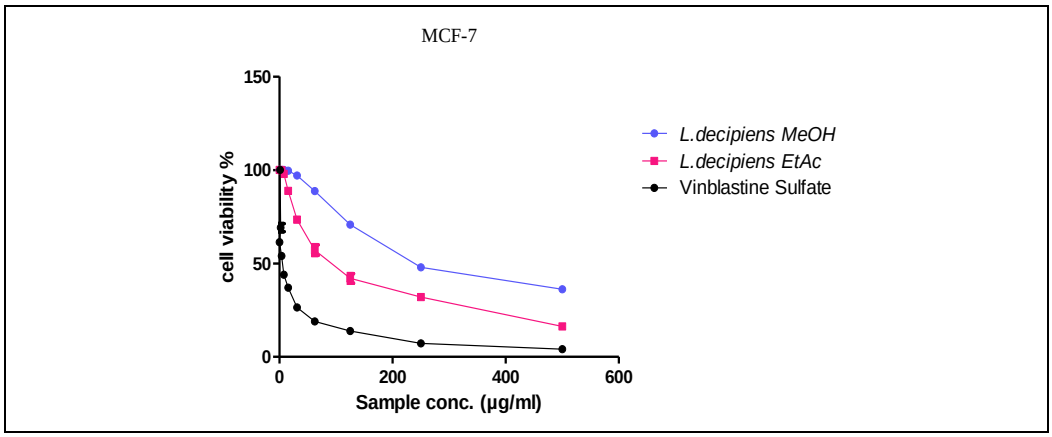



**Figure 5. cytotoxic activity (viability %) of methanol extract and ethyl acetate fraction of *L. decipiens* leaves on MCF-7 cell line compared to Vinblastine Sulfate**

(C-5″), 78.00 (C-2″),76.29 (C-3″), 71.66 (C-4″), 61.91 (C-6″), 56.83 ($OCH_3$).

Based on its chromatographic properties, $^1H$ NMR and $^{13}C$ NMR, compound **5** was expected to be spin coupling system of two ortho-doublets, each integrated for two Protons at δ ppm 7.67 (2H, d, *J* = 8.47 Hz, H-2′/6′) and 6.94 (2H, d, *J* = 8.69Hz, H-3′/5′) indicated 4′-hydroxyl. The glycoside moiety in compound **5** was identified as 3-*O*- glycoside from doublet signal at δ 5.38 (1H, d, *J* = 7.66Hz, H-1″) with *J* value 7.5Hz. Absence of H-6 signal from $^1H$ NMR spectrum of compound **5** and presence of singlet signal at 3.82 (3H, s, $OCH_3$ at C-6), together with$^{13}C$- NMR signal at δ ppm 56.83 of C- ($OCH_3$) has led to conclude that $OCH_3$must be on C-6, this evidence was confirmed by the downfield shift of C-6 to 128.94 (~ + 20 ppm) in $^{13}C$ NMR spectrum, which has also shown well resolved typical 15 signals of kaempferol-3-*O*-glucopyranoside, The down field shift of C-3 to 133.93 was confirmative evidence for the *O*-glycosylation at C-3. Moreover, the *O*-glycoside moiety in the structures was confirmed as 3-*O*-glucopyranoside depending on the characteristic down field location of C-1″ at 102.27 δ ppm with respect to those of C-glycosides. The assignment of all other $^{13}C$ NMR resonances of compound **5** was achieved by comparison with the corresponding data of structural related compounds[24,25]. According to the above discussed data compound **5** was confirmed as 6-(*O*-methyl)-kaempferol-3-*O*-glucopyranoside, which was isolated for the first time from *L. decipiens.*

**Compounds 6 and 7:** Obtained as yellow amorphous powder each, compound **6** (17 mg) and compound **7** (26 mg) chromatographic properties, $R_f$-values (0.43 $S_1$), (0.39 $S_2$) and (0.29 $S_1$), (0.21 $S_2$) respectively, they gave dark purple spot under UV-light, turned to yellow on exposure to $NH_3$ vapors, orange fluorescence on exposure to Naturstoff and green color with $FeCl_3$ spray reagents, Compound **6** and **7** were expected to be a luteolin structure[9].$^1H$ NMR spectra (**Table 1**) showed an ABX-spin coupling system of three proton resonances in each compound at δ 7.44 (1H, d, *J* = 2.4 & 8.25 Hz, H-6′), 7.41 (1H, d, *J* = 2.4 Hz, H-2′) and ortho-doublet at δ 6.90 (1H, d, *J* = 8.25 Hz, H-5′) for Compound **6** and δ 7.54 (1H, d, *J* = 2.4 & 8.97 Hz, H-6′), 7.49 (1H, d, *J* = 2.4 Hz, H-2′) and ortho-doublet at δ 6.86 (1H, d, *J* = 8.97 Hz, H-5′) for Compound **7** to indicate a 3′,4′ dihydroxy B-ring. in the aliphatic region, the doublets at δ 4.59 and 4.69 with large *J* values (> 9 Hz) for the anomeric protons of Compound **6** and Compound **7** respectively, were intrinsic for a *β*-C-glycoside moiety in both compounds. Absence of H-6 and H-8 signals from $^1H$ NMR of Compound 6 and Compound **7** respectively, was suggesting that the C-glycosylation must be on C-6 in case of Compound **6** and C-8 in case of Compound **7**. kaempferol 3-*O*-glucoside structure[24]. This expectation was supported by $^1H$ NMR spectrum showing an $A_2X_2$

As further confirmation, $^{13}C$ NMR spectrum for each compound (**Table 1**) showed well-resolved typical 15 signals of a luteolin aglycone moiety, including the three key signals of C-3′, C-4′ and C-3 at δ ppm 146.14, 150.07 and 103.11 for compound 6 and at δ ppm 146.51, 150.04 and 102.75 for compound **7**. Additionally, the C-glycoside moiety in both structures was confirmed as *β*-glucopyranoside depending on the characteristic up-field location of C-1″ at 73.50 and 73.48 ppm for compound **6** and compound **7**, respectively, and downfield location of both C-5″ and C-3″ to δ 81.92 and 79.18 ppm for compound **6** and to δ 82.37 and 79.03 ppm for compound **7**, with respect to those of *O*-glycosides[19]. The C-glycosidation at C-6 in compound **6** and at C-8 in compound **7** was concluded from the downfield shift of $^{13}C$-signals of C-6 to 109.19 and of C-8 to δ 104.91(~ + 10 ppm) for compounds **6** and **7**, respectively. The assignment of all other $^{13}C$ NMR resonances of compounds **6** and **7** was achieved by comparison with the corresponding data of structural related compounds[14,19,26,27,29]. Thus according to the above discussed data, compound **6** was confirmed as luteolin-6-C-*β*-D-glucopyranoside (Isoorientin), while compound **7** was confirmed as luteolin-8-C-*β*-D-glucopyranoside (Orientin). However this is the second report of their isolation from *Livistona decipiens*[30].

**Antioxidant activity**

The antioxidant activity of both methanol extract and ethyl acetate fraction may be attributed to phenolic contents of this plant; mainly phenolic acids, flavonoids which has been shown in phytochemical screening. The ethyl acetate fraction has moderate activity with $SC_{50}$ = 23 ± 0.74 µg/ml when compared to ascorbic acid $SC_{50}$ = 14.2 ±0.35 µg/ml, while the methanol extract has lower activity with $SC_{50}$ =55.2 ± 1.9 µg/*ml* (**Table 2** and **Figure 2**)

**Antimicrobial activity**

**Table 3** showed that the ethyl acetate fraction possess moderate activity against Gram positive and Gram negatvie bacteria,while the methanol extract has shown no action on Gram negatvie bacteria, however both of them possess no anti-fungal activity.

**Evaluation of cytotoxicity**

Using the MTT assay the cytotoxic effect of the tested samples on MCF-7, HeLa and HepG2 cell lines were shown in **Figure 3, 4** and **5** as well as in tables (4, 5 and 6).Results showed that *Livistona decipiens* methanol extract and ethyl acetate fraction have cytotoxic activity against the three cell lines. Ethyl

acetate fraction have moderate cytotoxic activity on the MCF-7 and HepG2 cell lines, with $IC_{50}$ = 91.9 ± 15.2 µg/ml and $IC_{50}$ =75.7 ± 11.86 µg/ml respectively, while it has a lower cytotoxic activity on HeLa cell lines with $IC_{50}$ =118 ± 4.5 µg/ml when compared to vinblastine sulfate with $IC_{50}$ = 5.44 ± 0.57 µg/ml, $IC_{50}$ = 3.48 ± 0.22 µg/ml and $IC_{50}$ = 6.54 ± 0.39 µg/ml, respectively, while, *L. decipiens*methanol extract have lower cytotoxic activity (**Table 7**).

## CONCLUSION

Seven compounds were isolated from the ethyl acetate and *n*-butanol fractions of *L. decipiens leaves*, for the first time from this species, *vies;*apigenin-8-C-*β*-D-glucopyranoside (Vitexin), Quercetin-6-C-*β*-D-glucopyranoside,apigenin-6,8-di-C-β-D-glucopyranoside (Vicenin II), 6-*O*-methyl Kaempferol 3-*O*-glucopyranoside, luteolin-3-C-gentiobiosyl,luteolin-6-C-*β*-D-glucopyranoside (Isoorientin) and luteolin-8-C-*β*-D-glucopyranoside (Orientin) The ethyl acetate fraction has shown moderate activity against Gram positive and Gram negatvie bacteria, also it showed moderate antioxidant activity when compared to ascorbic acid. Ethyl acetate fraction has shown moderate cytotoxic activity on MCF-7 cells, HepG-2 and lower activity on HeLa cells.

### Conflict of Interest

The authors declare that they do not have any conflict of interest.

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