

Studies on antimicrobial, antioxidant and phytochemical analysis of *Urena lobata* Leave extract

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Abstract

The leaves extracts of *Urena lobata* L were examined for their antioxidant, antibacterial and antifungal activities. Preliminary evaluation of both the crude and the solvent fractions showed a broad spectrum of activity since the extracts inhibit the growth of both gram positive and gram negative bacterial isolates. The ethyl acetate and *n*-butanol fractions had a fast antioxidant reaction with DPPH solution, while the *n*-hexane and dichloromethane fractions gave no reaction. Three compounds were isolated from the ethyl acetate fraction and their structures determined, on the basis of spectroscopic data, to be kaempferol **1**, quercetin **2**, and 3-O- β -D-(6''-O-*trans*-*p*-coumaroyl)- α -L-glucopyranosyl-kaempferol **3** (tiliroside). The compounds showed strong antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Bacillus polyxyma* and *Candida albicans*. The compounds also showed moderate to fast radical scavenging properties against DPPH radical. It was concluded that the isolated flavanoids may be part of the compounds responsible for the biological activity of *Urena lobata* leaf extract. This study therefore supports the traditional uses of the plant in the treatment of infectious diseases.

Key words: *Urena lobata*, Malvaceae, antimicrobial, antioxidant activities, flavonoids, NMR spectroscopy.

Introduction

Urena lobata Linn (Malvaceae), otherwise called Caesar weed, is a shrub that grows between 0.6- 3 m tall and up to 7 cm in basal diameter (Bautista, 2000; Uphof, 1959). Various extracts of the leaves and roots are used in herbal medicine to treat diverse ailments such as cough, malaria, venereal diseases, wounds, toothache, rheumatism and as abortifacient (De Las Heras et al., 1998; Francis, 2003). The leaves and flowers are eaten as famine food in Africa, and a semi-purified glycoside obtained from *Urena lobata* leaves was 86 percent as effective as aspirin in rats (Bautista, 2000). Previous research by other workers on the aerial part of the plant (Srinivasan et al., 1981) yielded mangiferin and quercetin. Recently, imperatorin, a furocoumarin was isolated from the roots (Keshab, 2004). From the ethyl acetate fraction of the leave, we now report the isolation of three flavonoid compounds together with the biological and radical scavenging properties.

Experimental Section

Melting points were determined on Gallenkamp electrothermal melting point apparatus. The Infrared (IR) spectra were run using KBr disc or nujol. The ^1H and ^{13}C Nuclear Magnetic Resonance spectra

were run on Varian Mercury spectrometer operating at 200 MHz for ^1H and 50 MHz for ^{13}C . The chemical shift values were reported in ppm relative to TMS as internal standard. All solvents used for extraction and column chromatography were General Purpose Reagents (GPR), redistilled before use. Gel filtration was performed using Sephadex LH-20 previously swollen in specified solvent(s) prior to loading of extract onto the column (3.5 cm x 8.5 cm). Thin layer chromatography (tlc) were done with aluminium sheet pre-coated with normal phase silica gel 60 F₂₅₄ (Merck, 0.20 mm thickness). The tlc plate analyses were run using any of the following solvent systems: Solvent system A: *n*-hexane–ethyl acetate (3:1); Solvent system B: chloroform – ethyl acetate (9:1); Solvent system C: dichloromethane – ethyl acetate (4:1); Solvent system D: dichloromethane – ethyl acetate – methanol (1:9:1); Solvent system E: chloroform – ethyl acetate (1:9) and Solvent system F: ethyl acetate – methanol (9:1). Spots were located on the developed tlc plates by viewing under ultraviolet light (254 nm and 366 nm) and then sprayed with any of the following detecting reagents: methanolic solution of ferric chloride (5 g/100 ml), vanillin-sulphuric acid (0.2 g/100 ml); 0.1 % solution of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) in methanol, and Dragendorff's reagent, tlc plates sprayed with vanillin-sulphuric acid were activated by heating at 110 °C for 2 minutes.

Plant Material

The leaves of *Urena lobata* Linn were collected along Mayfair road, Ile-Ife, Osun state, Nigeria in August 2005. The plant was identified by Dr. H.C. Illoh of the Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife. A voucher specimen No. FHI 15352 was deposited in the herbarium of the same department.

Extraction and Isolation

A 2.2 Kg air-dried powdered leaves of *Urena lobata* was extracted at room temperature with 50 % aqueous-ethanol for 72 hours. The resulting mixture was filtered and then concentrated to dryness *in vacuo* on a rotary evaporator. The crude extract obtained, coded as CLUL, was re-dissolved in water and successively partitioned with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. The solvent extracts obtained were concentrated to dryness on a rotary evaporator and then screened for biological activities as described under the sections – antimicrobial and antioxidant assay. The ethyl acetate fraction was found to be most active against certain strains of bacterial isolates and as well showed a very fast antioxidant reaction with DPPH solution. The ethyl acetate extract (4.90 g) was then subjected to bioactivity-guided fractionation using the accelerated gradient chromatography (AGC) separation technique with solvent combination of *n*-hexane as starting solvent and a sequential increase in polarity by volume between *n*-hexane, dichloromethane, ethyl acetate and methanol. A total of 165 test tube fractions (15 ml each) were collected and analyzed by tlc using any of the solvent systems (A-F). Fractions showing similar tlc characteristics were pooled together and concentrated to dryness *in vacuo* on a rotary evaporator. Ten different fractions coded as UL₅A (83 mg), UL₅B (231 mg), UL₅C (91 mg), UL₅D (19 mg), UL₅E (59 mg), UL₅F (192 mg), UL₅G (302 mg), UL₅H (697 mg), UL₅I (674 mg) and UL₅J (2.1 g) were obtained. *In vitro* tlc antioxidant test was carried out on all fractions. However, a detailed column chromatographic fractionation using LH-20 Sephadex (50% toluene-ethanol with gradient increase in polarity) and accelerated gradient chromatography on fractions UL₅G (274 mg), UL₅H (660 mg) and UL₅I (630 mg) resulted in the isolation of three compounds. The structures of the three compounds were established on the basis of the spectroscopic data including COSY, ^1H , ^{13}C , HMBC, APT and IR.

Antimicrobial assay

The sensitivity testing of the plant extracts were determined using agar-well diffusion method as described by Irobi et al., (1996). Nutrient broth (Oxoid Ltd) and nutrient agar (Oxoid Ltd) were used for sub-culturing the bacterial isolates, while diagnostic sensitivity test agar (Oxoid Ltd) was used for sensitivity testing. The organisms used were of the National Collection of Industrial Bacteria (NCIB) and some Locally Isolated Organism (LIO). They were obtained from the stock culture of the Department of Microbiology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria. The bacterial isolates were first grown in nutrient broth (Oxoid Ltd) for 18 hours before use. The inoculum

suspension were standardized and then tested against the effect of the extracts at a concentration of 20 mg/ml each (for the crude and solvent extracts) and 2 mg/ml each (for the isolated compounds from the ethyl acetate extract) in diagnostic sensitivity test agar (Oxoid Ltd). Water-methanol mixture was used as solvent of dissolution of various extracts and control. The plates were observed for zones of inhibition after 24 hours incubation at 37 °C. The effects were compared with streptomycin (standard antibiotic) at a concentration of 1 mg/ml. The diameters of the zones of inhibition were measured to the nearest millimeter.

Antioxidant assay

The organic solvent extracts obtained from the crude aqueous-ethanol extract of *Urena lobata* leaves (*n*-hexane, dichloromethane, ethyl acetate and *n*-butanol) were spotted on aluminum coated tlc silica gel plates and developed in different solvent systems in a chromatography tank. The *n*-hexane fraction was best separated in solvent system A (*n*-hexane-ethyl acetate, 3:1); the dichloromethane fraction separated well in solvent systems B and C (chloroform-ethyl acetate, 9:1, and dichloromethane-ethyl acetate, 4:1); the ethyl acetate fraction was run in solvent system D and E (dichloromethane-ethyl acetate-methanol, 1:9:1, and chloroform-ethyl acetate 1:9), while the *n*-butanol fraction gave appreciable separation in solvent system F (ethyl acetate-methanol, 9:1). The plates were removed from the tank, air-dried, and then viewed under the UV light at 254 and 366 nm, respectively. The dried tlc plates were then sprayed with a 0.1 % w/v solution of the stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol. Spots with antioxidant activity inhibit free radical effect of DPPH thus leading to deep yellow colour on a purple background on the tlc plates. The antioxidant activity of the various spots was monitored based on the colour changes observed with time in seconds on the tlc plates. (Cuendet et.al., 1999; 2000)

Phytochemical analysis and determination of pH value

Test for Alkaloids: A modified form of the thin-layer chromatography (TLC) method described by Farnsworth and Euler (1962) was used. One gramme of the extract was treated with 40 per cent calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10 ml portions of chloroform. The extract was then spotted on the thin-layer plates. Solvent systems A and B were used as mobile phase. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatogram with freshly prepared Dragendorff's spray reagent. A positive reaction on the chromatogram (indicated by orange or darker-coloured spot against a pale yellow background) was confirmatory evidence that the plant extract contained an alkaloid.

Test for Tannins: About 2.5 g of the plant extract was dissolved in 5 ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins (Trease and Evans, 1989).

Determination of pH value: The pH was measured using a pH meter of a glass electrode. The glass electrode was immersed in water for several hours before use. The measurement started about 5 minutes after the equipment was switched on and the detecting unit was rinsed well with water and cleaned with a piece of filter paper.

The pH meter was adjusted at one pH value and the temperature compensation dial was rotated to set the temperature of the pH standard solution. The detecting unit was then immersed in the pH standard solution and measurement taken about 2 minutes when the pH meter is set to the pH of the standard solution.

The detecting unit was removed from the standard solution, washed well with water and gently blotted with a piece of filter paper to remove water. It was then immersed in the sample solution, and the pH value measured. The temperature of the sample solution and that of the pH standard solution was the same.

Results and Discussion

Preliminary phytochemical screening of the crude extract of *Urena lobata* showed the presence of phenolic compounds (as shown by strong reaction with ferric chloride) but no reaction with Draggendoff's reagent indicating the absence of alkaloids in the plant extract. The pH value of the crude extract was determined and found to tend towards neutrality. This is an indication that the antimicrobial activity of the plant extract was not affected by the pH.

Table 1 shows the antimicrobial activity of crude extract and solvent fractions of the *Urena lobata* leave. The crude extract had a broad spectrum of activity against both the gram-positive and gram-negative bacterial isolates. However, it was observed that the activity was extractable into the different fractions with the ethyl acetate fraction being the most active extract against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium sporogenes* and *Candida albicans*. The *n*-butanol fraction only showed higher activity against *Pseudomonas aeruginosa*, while *n*-hexane fraction had stronger activity against *Bacillus stearothermophilus*, *Streptococcus faecalis*, *Bacillus polyxyrna* and *Klebsiella pneumoniae*. The dichloromethane exhibited the least antimicrobial activity as compared to other four fractions.

Table 1 Antimicrobial activity of Crude Extract and Solvent fractions of Leaf *Urena lobata* Linn.
Mean zones of Inhibition (mm) *

Organisms	Sample 1 (20 mg/ml)	Sample 2 (20 mg/ml)	Sample 3 (20 mg/ml)	Sample 4 (20 mg/ml)	Sample 5 (20 mg/ml)	Streptomycin (1 mg/ml)
<i>Pseudomonas aeruginosa</i> (NCIB 950)	15	14	13	18	20	21
<i>Bacillus cereus</i> (NCIB 6349)	15	20	20	24	18	28
<i>Serratia marcescens</i> (NCIB 1377)	18	16	17	20	16	19
<i>Bacillus stearothermophilus</i> (NCIB 8222)	15	28	20	26	14	23
<i>Escherichia coli</i> (NCIB 86)	27	24	28	32	25	0
<i>Pseudomonas fluorescens</i> (NCIB 3756)	12	12	14	15	13	30
<i>Shigella dysenteriae</i> (LIO)	15	13	16	12	16	22
<i>Staphylococcus aureus</i> (NCIB 8588)	12	15	16	18	17	21
<i>Streptococcus faecalis</i> (NCIB 775)	15	18	15	17	13	23
<i>Corynebacterium pyogenes</i> (LIO)	15	18	14	18	13	20
<i>Bacillus polyxyrna</i> (LIO)	12	20	14	17	14	15
<i>Clostridium sporogenes</i> (LIO)	15	17	22	30	16	25
<i>Bacillus subtilis</i> (NCIB 3610)	15	20	18	25	18	20
<i>Klebsiella pneumoniae</i> (NCIB 418)	20	22	16	18	20	0
<i>Bacillus anthracis</i> (LIO)	12	11	10	13	12	18
<i>Candida albicans</i>	24	26	24	26	23	ND

Keys: (mm)* = Mean diameter of three replicates; Sample Codes: **1** = Crude Extract of leaf; **2** = *n*-hexane fraction; **3** = dichloromethane fraction; **4** = ethyl acetate fraction and **5** = *n*- butanol fraction. ND =Not Done.

Table 2 shows the antimicrobial activity of the fractions obtained from the accelerated gradient chromatographic (AGC) column of the ethyl acetate fraction.

Table 2

Organisms		Mean Zones of Inhibition (mm)*					Streptomycin (1 mg/ml)
		₅ (A-F) (20 mg/ml)	₅ G (20 mg/ml)	₅ H (20 mg/ml)	₅ I (20 mg/ml)	₅ J (20 mg/ml)	
<i>Pseudomonas aeruginosa</i> (NCIB 950)		17	15	12	0	nt	21
<i>Bacillus cereus</i> (NCIB 6349)		15	14	13	12	nt	28
<i>Serratia marcescens</i> (NCIB 1377)		13	18	14	16	nt	19
<i>Bacillus stearotherophilus</i> (NCIB 8222)		13	25	23	15	nt	23
<i>Escherichia coli</i> (NCIB 86)		15	25	23	25	nt	0
<i>Pseudomonas fluorescens</i> (NCIB 3756)		16	14	17	15	nt	30
<i>Shigella dysenteriae</i> (LIO)		15	17	14	16	nt	22
<i>Staphylococcus aureus</i> (NCIB 8588)		18	14	16	16	nt	21
<i>Streptococcus faecalis</i> (NCIB 775)		15	13	15	17	nt	23
<i>Corynebacterium pyogenes</i> (LIO)		16	14	17	15	nt	20
<i>Bacillus polyxyma</i> (LIO)		15	15	17	16	nt	15
<i>Clostridium sporogenes</i> (LIO)		18	16	15	17	nt	25
<i>Bacillus subtilis</i> (NCIB 3610)		15	28	26	28	nt	20
<i>Klebsiella pneumoniae</i> (NCIB 418)		15	15	18	16	nt	0
<i>Bacillus anthracis</i> (LIO)		14	12	15	15	nt	18
<i>Candida albicans</i>		23	25	26	28	nt	ND

Keys:

(mm)* = Mean diameter of three replicates. Sample codes: UL₅ (A-F) (Tubes: 1- 109); UL₅G (Tubes: 110-114); UL₅H (Tubes: 115-127); UL₅I (Tubes: 128-140); UL₅J (Tubes 141-160). nt = not tested.

A preliminary *in vitro* antioxidant test showed that only ethyl acetate and *n*-butanol fractions had fast reaction with solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) 98 % free radical reagent in time ranging between 0 – 5 minutes, while dichloromethane fraction recorded between 10 – 30 minutes and no reaction with *n*-hexane fraction. It is on the basis of these results that the ethyl acetate fraction was chosen for detailed study. Table 3 shows the result of the bioactivity-guided chromatographic fractionation of the ethyl acetate fraction monitored by rapid tlc spray with DPPH solution afforded ten different sub-fractions coded as UL₅A- UL₅J. The fractions UL₅A – UL₅F showed very weak antioxidant reaction with DPPH; hence they were pooled together as a fraction for antimicrobial test. However, fractions UL₅G, UL₅H and UL₅I eluted with different solvent ratio combination of dichloromethane- ethyl acetate and methanol had a fast reaction with DPPH solution (Table 3).

Table 3: *In-vitro* tlc antioxidant studies of fractions AGC fractions of ethyl acetate extract.

Fraction(s) UL	₅ A – ₅ F	₅ G	₅ H	₅ I	₅ J
Intensity	Low	High	High	High	moderate
Time of Antioxidant spot Development.	Over 30 minutes	1-5 seconds	1-5 seconds	1- 10 seconds	1-15 seconds

Table 4 shows that the isolated compounds had varied antimicrobial activity against selected bacterial isolates. TLC analysis of the compounds also showed fast *in vitro* antioxidant reactions with 1, 1-diphenyl-2-picrylhydrazyl (DPPH) solution with time ranging between 0- 5 seconds; this is expected because of the presence of poly-hydroxyl moieties in the molecules. Ascorbic acid is used as reference standard for the test (Cuendet et al., 1997; Min Zhu, et al., 1999).

Table 4: Antimicrobial activity of isolated flavonoid compounds from EtOAc extract of leaf (*Urena lobata*).

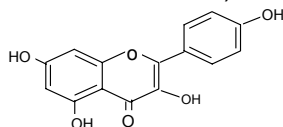
Organisms	Mean Zones of Inhibition (mm)*			Streptomycin (1 mg/ml)
	Compound 1 2 mg/ml	Compound 2 2 mg/ml	Compound 3 2 mg/ml	
<i>Pseudomonas aeruginosa</i>	18	14	13	21
<i>Bacillus cereus</i>	24	20	20	28
<i>Serratia marcescens</i>	18	16	17	19
<i>Bacillus stearothermophilus</i>	26	28	20	23
<i>Escherichia coli</i>	32	24	28	0
<i>Pseudomonas fluorescens</i>	15	16	14	30
<i>Shigella. dysenteriae</i>	16	16	18	22
<i>Staphylococcus aureus</i>	15	14	17	25
<i>Streptococcus faecalis</i>	18	18	12	23
<i>Corynebacterium pyogenes</i>	18	18	15	20
<i>Bacillus polyxyrna</i>	17	20	14	15
<i>Clostridium sporogenes</i>	30	17	15	25
<i>Bacillus subtilis</i>	25	20	18	20
<i>Klebsiella pneumoniae</i>	19	22	16	0
<i>Bacillus anthracis</i>	17	15	15	18
<i>Candida albicans</i>	26	26	24	ND

Keys:

(mm)* = Mean diameter of three readings. ND = Not Done

Spectroscopic and physicochemical data.

Compound 1 (14 mg): Obtained from fraction UL₅G. Yellow solid, shows deep intense colour with vanillin-sulphuric acid reagent. R_f values: 100% EtOAc (0.86); EtOAc – Chloroform 9:1 (0.78), and Dichloromethane-EtOAc 4:1 (0.76). The structure was established by ¹H, ¹³C NMR, COSY and HETCOR. The proton nmr spectrum of the compound gave four proton doublet peaks at the aromatic region. The aromatic proton doublets at δ 6.17 and δ 6.38 were due to the meta-coupled protons of a 5, 7- substituted ring A, this accounted for protons H-6 and H-8 respectively. The two proton doublets at δ 6.89 (d, J = 9.0 Hz, 2H) and δ 8.08 (d, J = 9.0 Hz, 2H) accounted for the protons H- 2', 6' and H – 3', 5' of a 4'-substituted ring B respectively. ¹H and ¹³C nmr spectra of Compound 1 is consistent with those published for kaempferol (Markham et. al., 1978).

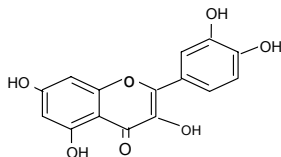


Structure of compound 1

IR (Nujol mull) vcm-1 3314 [OH], 2905 [C-H], 1732 [C=O], 1657 [C=C], 1612,1567,1505,1459, 1380 and 1230. ¹H NMR CD₃OD (δppm): δ 6.17 (1H, d, J = 1.8 Hz, H – 6), δ 6.38 (1H, d, J = 1.8 Hz, H- 8); δ 6.89 (2H, d, J = 9.2 Hz, H – 2', 6'); δ 8.08 (2H, d, J = 8.8 Hz, H – 3', 5'). ¹³C NMR (CD₃OD): δ 94.4

(C-8), 99.3 (C-6), 104.5 (C-10), 116.3 (C-3', 5'), 123.7 (C-1'), 130.7 (C-2', 6'), 137.2 (C-3), 148.0 (C-9), 158.2 (C-2), 160.6 (C-5), 162.5 (C-7), 165.6 (C-4') and 177.4 (C-4).

Compound **2** (15 mg): Obtained from fraction UL₅H, a deep yellow solid. R_f values: 0.70 EtOAc - chloroform (4:1), 0.33 chloroform- EtOAc (9:1). The proton nmr spectrum showed 5 aromatic signals. The aromatic proton doublets at δ 6.25 and 6.50 ppm ($J = 1.8$ Hz) were due to the meta-coupled protons of a 5, 7- substituted ring A, for protons H-6 and H-8 respectively, while the signals at δ 6.98 (d, $J = 8.4$ Hz, 1H), δ 7.66 (dd, $J = 1.8, 8.4$ Hz, 1H) and δ 7.79 (d, $J = 1.8$ Hz, 1H) were assigned for protons H-5', H-6' and H-2', showing an ABX coupling system of a 3', 4'- substituted ring B of a flavanol. Confirmation of the structure was by comparison of the ¹H nmr of compound **2** with the literature values, (Markham and Ternai, 1978). The literature revealed compound **2** to be quercetin, a flavonoid that is widely distributed in the plant kingdom (Wollenmweber and Dietz, 1981).

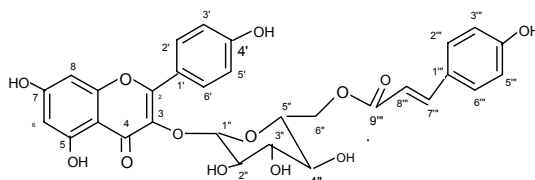


Structure of compound **2**

¹H NMR: δ 6.25 (1H, d, $J = 1.5$ Hz, H - 6); δ 6.50 (1H, d, $J = 1.8$ Hz, H - 8); δ 6.98 (1H, d, $J = 8.4$ Hz, H - 5'); δ 7.66 (1H, dd, $J = 1.8$ Hz, 8.4 Hz, H - 6'); δ 7.79 (1H, d, $J = 1.8$ Hz, H - 2'); δ 12.18 (br, 5-OH).

Compound **3** (34 mg): Obtained from fraction UL₅I, a bright yellow solid; R_f value of 0.49 in dichloromethane- EtOAc -methanol (1:9:1). Melting point = 206-208 °C. IR spectrum showed the following absorption bands: Hydroxyl (3120 cm⁻¹), α , β - unsaturated C=O (1627 cm⁻¹), carbonyl (1727 cm⁻¹), and aromatic (1628, 1584, 1539 and 1460 cm⁻¹) functions. ¹H nmr showed coupling system in the aromatic region between protons at δ 7.98 (d, $J = 8.8$ Hz, 2H) and 6.87 (d, $J = 8.8$ Hz, 2H) characteristic of a 4'- substituted ring B. These signals accounted for the protons H-2', 6' and H-3', 5' of ring B. The aromatic proton doublets at δ 6.18 (d, $J = 2.0$ Hz, 1H), and δ 6.38 (d, $J = 2.0$ Hz, 1H) were assigned to a meta-coupled protons of a 5, 7-substituted ring A for protons H-6 and H-8 respectively. The glycosidic nature was indicated by multiplet peaks at δ 4.05 – 4.30 ppm in the ¹H nmr spectrum. The sugar moiety was inferred to be glucose from a doublet signal at δ 5.18 ppm integrating for two protons of methylene as well as the ¹³C nmr signal at δ 63.3 ppm. The other glycosidic carbon signals were found between δ 70 – 76 ppm, while the anomeric carbon 1'' was at δ 101.2 ppm. The attachment of the glucose unit was determined to be C-3 (OH) group of the aglycone. Other peaks on the proton nmr include a *trans* olefinic protons at δ 6.08 and 7.34 ppm and 2 pairs of *ortho*-coupled aromatic protons δ 6.78 and 7.36 ppm. The acyl unit was identified as *trans-p*-coumaric acid. The site of esterification was determined to be C-6'' position of the glucose moiety. ¹H and ¹³C nmr spectral of Compound **3** were identical with those published for tiliroside (Backhouse *et al.*, 2002).

¹H NMR (DMSO d₆): δ 6.18 (1H, d, $J = 2.0$ Hz, H-6); δ 6.38 (1H, d, $J = 2.0$ Hz, H-8); δ 7.98 (2H, d, $J = 8.8$ Hz, H-2', 6'); δ 6.87 (2H, d, $J = 8.8$ Hz, H-3', 5'); δ 5.48 (1H, d, $J = 7.8$ Hz, H-1''); δ 7.37 (1H, d, $J = 8.6$ Hz, H-2'''); δ 7.55 (1H, d, $J = 8.7$ Hz, H-6'''); δ 6.70 (1H, d, $J = 8.6$ Hz, H-3'''); δ 6.58 (1H, d, $J = 8.9$ Hz, H-5'''); δ 7.34 (1H, d, $J = 15.9$ Hz, H-7'''); δ 6.69 (1H, d, $J = 17.9$ Hz, H-8'''). ¹³C NMR (DMSO d₆): δ 156.7 (C-2), 133.4 (C-3), 177.7 (C-4), 161.2 (C-5), 99.1 (C-6), 164.4 (C-7), 94.1 (C-8), 159.9 (C-9), 104.2 (C-10), 121.2 (C-1'), 131.5, 130.5 (C-2', 6'), 115.1, 115.4 (C-3', 5'), 160.2 (C-4'), 101.2 (C-1''), 74.4 (C-2''), 76.4 (C-3''), 70.2 (C-4''), 74.5 (C-5''), 63.3 (C-6''), 125.7 (C-1'''), 131.2, 131.2 (C-2''', 6'''), 115.9, 116.6 (C-3''', 5'''), 160.2 (C-4'''), 145.0 (C-7'''), 113.9 (C-8''') and 166.9 (C-9''').



Structure of Compound **3** (Tiliroside)

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