Publications


Seminars


Presented a paper entitled “Comparison of cardenolide principle in Digitalis purpurea and Premna serratifolia.” in International seminar on World Bioheritage concerns over climate change, with a special reference to ethnic vegetables held at Sree Narayana College, Chempazhanthy, Thiruvananthapuram on September 7th and 8th, 2012.

Presented a paper titled “Anatomical and Phytochemical characterization of the roots of Premna serratifolia L.” in International Seminar on Molecular secrets of Plant Medicines (MSPM 2013) held at C.M.S. College, Kottayam on 17th and 18th October 2013.

Achievements

Received Certificate of Merit in P. D. Sethi Research paper Annual award 2010 on application of TLC/HPTLC in Pharma, Herbal and Miscellaneous Analysis for the paper entitled “Investigative study of Novel cardiac glycoside in the root of Premna serratifolia L. with Digoxin using HPTLC.”

The paper titled “Anatomical and Phytochemical characterization of the roots of Premna serratifolia L.” was selected as the best paper in International Seminar on Molecular secrets of Plant Medicines (MSPM 2013) held at C.M.S. College, Kottayam on 17th and 18th October 2013.
Identification of novel cardiac principle in the roots of *Premna serratifolia* L.

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**ABSTRACT**

Cardiac glycoside in minute dosage is well recognized as a medicine for the treatment of congestive heart failure. Among the medicinal plants, *Digitalis purpurea* is known as a natural source of vital cardiac glycosides. *Premna serratifolia* L. (synonyms: *Premna integrifolia* L., *Premna obtusifolia* R. Br.; family: Verbenaceae) is an essential ingredient of many imperative ayurvedic drugs. Indigenous knowledge on *P. serratifolia*, regarding its cardio-tonic property, is suggestive of certain novel cardiac principles in it. Hence, a foremost exertion to identify cardiac glycoside principle in its roots has been undertaken by HPTLC, using *Digitalis purpurea* and digoxin as positive standards. The detection of an identical compound like that of digoxin, in the roots of *P. serratifolia*, justifies its indigenous medicinal property as an effective cardio-tonic drug. Further studies are needed to find out the structural identity of this newly reported cardiac principle.

**Key words:** *Premna serratifolia*, Digoxin, *Digitalis purpurea*, HPTLC, Cardiac glycoside.

**INTRODUCTION**

Heart failure is an extremely severe cardiac state associated with a high mortality rate. Pharmacological therapies for heart failure include usage of diuretics, angiotensin-converting enzyme inhibitors, stimulation by beta-blockers, usage of vasodilators, and direct augmentation of depressed cardiac contractility with positive isotropic drugs. Among cardiac medicines, cardiac glycosides have been clinically accepted for the treatment of heart failure and atrial arrhythmia⁴. The use of medicinal plants containing cardiac glycoside can be dated back to 1500 years. They have been traditionally used as arrow poisons, abortifacients, emetics, diuretics, and heart tonics. Structurally, all cardiac glycosides include a steroid nucleus and a lactone ring, usually having one or more sugar residues⁴.

The cardiac glycosides are often called ‘digitalis’ because many of them are extracted from the Foxglove plant, *Digitalis purpurea*, belonging to the family, Scrophulariaceae⁵. The cardiac glycoside extracted from this plant contains two cardenolide compounds viz., digitoxin and digoxin. Both of these cardenolide compounds act primarily on the heart. Pure digoxin is odorless, white crystals, insoluble in water. This drug has been used for over two centuries to treat congestive heart failure. It is normally used in serum concentrations of 0.8 to 2.0 µg/ml. It works by inhibiting sodium-potassium adenosine triphosphatase inside the cells of the heart. It helps to regulate the heart beat and makes the heart beat stronger. In short, the benefits of digoxin are greatest in patients with severe heart failure, an enlarged heart and a third heart beat and makes the heart beat stronger. In short, the benefits of digoxin are greatest in patients with severe heart failure, an enlarged heart and a third heart sound gallop.

*Premna serratifolia* L. (synonyms: *Premna integrifolia* L., *Premna obtusifolia* R. Br.; family: Verbenaceae) is a promising medicinal plant as a cardio-protective agent. It is an aromatic shrub with greenish yellow/white flowers and light brown / yellowish brown woody aromatic roots. Its root is an active constituent of *dasamoola* and *brahat panchamoola*. Its root, bark and leaves are reported to have many indigenous medicinal uses to tune up the functioning of respiratory, digestive, nervous and cardio-vascular systems of the body. The traditional vaidyans of Kerala use the roots of this plant as a mild cardiac stimulant⁶. Recent studies confirmed the effectiveness of the ethanol extract of stem-bark and stem-wood to prevent myocardial infarction based on histological changes and biochemical markers of cardiac tissue damage⁷,⁸.

It is speculated that the protective myocardial effect of ethanol extract of *P. serratifolia* L., on isoproterenol-induced myocardial infarction in rats may be due to its phytoconstituents like iridoid glycosides, alkaloids, flavonoids and phenolic compounds. It is also reported that the secondary metabolites in *P. serratifolia*, may cause constriction of blood vessels and may raise the blood pressure⁹. These findings suggest the possibility of having some compounds similar to that of digoxin in *P. serratifolia*. However, no research studies have so far been initiated to screen the cardio-protective constituents in the roots of *P. serratifolia* using authentic marker compounds. Hence, an attempt is made in the present investigation to identify the cardio-protective principle in the roots of *Premna serratifolia* by HPTLC using digoxin, as positive standard. The study also aims to quantify the cardiac glycoside principle in its roots by densitometric analysis.

**MATERIALS AND METHODS**

**Sample preparation**

*Premna serratifolia* L., growing in two different habitat conditions viz., non-saline and saline habitats were collected from two different localities. Fresh roots of *P. serratifolia* growing in non-saline habitats (Ecotype I) was collected from the botanical garden of C.M.S. College, Kottayam, Kerala and that of saline habitats (Ecotype II) from coastal region of Alappuzha, Kerala. The plants were authenticated and the voucher specimens are kept in CMS Herbarium, Kottayam, Kerala, 686001, India. The roots of collected plants were cleaned with fresh water, chopped, shade dried and coarsely powdered (40 mesh size) using an electric mixer. The powdered roots of *P. serratifolia* Ecotype I and Ecotype II (60 g. each) were extracted with 800ml 100% methanol in a soxhlet apparatus for 8 hrs⁴. The extract was then concentrated to 100 ml in a rotary vacuum evaporator. The concentrated extract was then used for qualitative analysis and for further separation of cardiac glyco-

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Phytochemical screening

The methanolic root extracts of Premana serratifolia (Ecotype I and Ecotype II) were subjected to qualitative analysis to detect the presence of cardiac glycosides [8, 9, 10].

HPTLC Method and Chromatographic Conditions

The HPTLC system (Camag, Muttenz, Switzerland) has Linomat V autosprayer connected to a nitrogen cylinder; a twin trough chamber (10×10 cm) and a derivatization chamber. Pre-coated silica gel 60 F254, E Merck) was used for spotting the samples. The derivatization chamber was pre-coated silica gel 60 (15 g) was extracted in 200 ml 100% methanol in a soxhlet apparatus for 8 hours[7]. The extract was then concentrated to 100 ml in a rotary vacuum evaporator. This extract was used as reference standard of digoxin.

Sample Application

The digoxin standard, its reference standard (methanolic extract of D. purpurea [15 g]) was extracted in 200 ml 100% methanol in a soxhlet apparatus for 8 hours[7]. The extract was then concentrated to 100 ml in a rotary vacuum evaporator. This extract was used as reference standard of digoxin.

RESULTS AND DISCUSSION

The phytochemical screening tests confirmed the presence of cardiac glycosides in the root extracts of both ecotypes of P. serratifolia. The results are tabulated in Table 1.

Table 1: Results of qualitative evaluation of cardiac glycosides in P. serratifolia roots

<table>
<thead>
<tr>
<th>Tests</th>
<th>P. serratifolia Ecotype I</th>
<th>P. serratifolia Ecotype II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keller-Killani Test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carr-Price Test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kedde’s Test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raymond’s Test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Legal’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Baljet’s Test</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The HPTLC profiles revealed the presence of a significant band in TLC at Rf 0.87, which matches with the standard digoxin, and that of its reference standard, Digitalis purpurea (Fig 1-4). The results of scanning of HPTLC plates (Table 2 and Graphs 2-5) revealed the presence of digoxin analogue in the methanolic root extracts of ecotype I (non saline) and ecotype II (saline) of P.serratifolia. A comparison of the spectra of the four tracks in HPTLC plate in the Rf0.87 region, corresponding to that of digoxin standard (Graph1) further confirmed this finding. As revealed in the scanning report, only mild concentration of this vital cardiac principle is detected in the roots of Premana serratifolia. However, its presence in slightly higher concentration in ecotype II indicates the role of salinity stress to boost the production of secondary metabolites in P.serratifolia.
The results of the present study revealed that the quantity of the digoxin-analogue compound in *P. serratifolia* is much less, when compared to its standard markers. The quantity of the root of *P. serratifolia* used for extraction is four times higher than the quantity of the *Digitalis purpurea* leaves taken for extraction. The recorded quantity of digoxin in *D. purpurea* leaves is 2 µg/µL, whereas that in *Premna serratifolia* ecotype I and II are 0.076 µg/
µL and 0.133 µg/µL respectively. Even at the higher concentrations, the presence of cardiac glycosides in the investigated plant roots (P. serratifolia—Ecotype I and Ecotype II) is of lesser quantity when compared to its standards i.e., digoxin or its counterpart mother plant extract of Digitalis purpurea. Even though the cardiac glycoside in Digitalis is widely acclaimed as the medicine for chronic heart diseases, it is not fully free from its toxicity. On the other hand, as revealed from the review of literature, P. serratifolia is described as a non-toxic cardiotonic agent capable of removing the obstruction of respiratory as well as cardiovascular systems. It is probable that the presence of cardiac glycoside compounds in moderate proportion provide the roots of P. serratifolia a mild and soothing cardio tonic property, without any side effects of its toxicity.

CONCLUSION
Clinical studies in heart failure patients have shown that digoxin, when used in conjunction with diuretics and vasodilators, improves cardiac output and ejection fraction and reduces filling pressures and pulmonary capillary wedge pressure by reducing pulmonary congestion and edema with very little changes in heart rate. This necessitates the search for novel cardiac principles in medicinal plants. In this context, an attempt is made to identify the cardiac principle in the roots of P. serratifolia using digoxin as positive standard. Considering the HPTLC profiles of the root extracts of the two ecotypes of P. serratifolia that matches well with the standard digoxin, further studies to characterize the active cardiac principle is well merited. The quantity of the cardiac glycoside compound in the two ecotypes of P. serratifolia had been compared. From the comparison, it is apparent that the roots collected from the saline habitat (Ecotype II) have higher concentration of cardiac glycoside than that of its counterpart in non saline habitat (Ecotype I). It is probable that saline stress factor induced the production of cardiac glycoside in Ecotype II. In short, the detection of an identical compound like that of digoxin, in the roots of P. serratifolia, justifies its indigenous medicinal property as an effective cardio-tonic drug.

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Identification of acteoside as the active antioxidant principle of *Premna serratifolia* root wood tissues

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**Abstract**

*Premna serratifolia* Linn. (syn: *Premna integrifolia*) is one of the most widely used plants in the Ayurvedic system of medicine. Several pharmacological activities including antioxidant effects and phytochemical investigations have been previously reported for the various parts of the plant, except the root woody tissues. In the present study, the antioxidant activity and active principle of the root woody tissues were investigated. Antioxidant effect was routinely monitored using the DPPH radical scavenging assay while phytochemical investigation was based on analysis using HPLC and Teledyne Isco flash chromatography system. Through the use of comprehensive spectroscopy studies, the isolated active antioxidant principle was identified as acteoside (verbacoside). Aceoside, which was about four times more active ($18.3 \pm 3.7 \mu g/ml; 11.4 \pm 2.3 \mu M$) than the crude root wood extract ($73.8 \pm 2.4 \mu g/ml$), could account for most of the reported pharmacological activity on *P. serratifolia*.

**Keywords:** *Premna serratifolia*; Verbenaceae; antioxidant; root wood; acteoside; verbacoside

**Introduction**

The genus *Premna* (Verbenaceae) is widely distributed in tropical and subtropical regions of Africa, Asia, Australia and the Pacific islands (Kadareit, 2004). *Premna serratifolia* Linn. (syn: *Premna integrifolia*), is a small tree or shrub that occurs in India, Srilanka and the Andaman. Various parts of the plant especially the roots are extensively used for thousands of years in traditional medicine formulations such as Dashmoola kwatha, Chyanprashavleh, Haritakiavleh, Ayushyavardhaak tel and Narayana taila (API, 2001; Jothi et al., 2010). Among the various medicinal uses reported for the roots are for treating diabetes, chyluria, gonorrhoea, inflammation, swelling, bronchitis, dyspepsia, headache, liver disorder, piles, constipation and fever (API, 2001). Some pharmacological studies have revealed that the plant posses anti-coagulant (Gopal and Purushothaman, 1984), anti-inflammatory (Karthikeyan and Deepa, 2011), anti-arthritis (Karthikeyan and Deepa, 2010; Rajendran and Krishnakum-
Yadav et al. (2011) have recently studied the antioxidant activity of the stem bark of *P. serratifolia* along with its active constituents. It was reported that 4″-hydroxy- E-globularinin, 10-O-trans-p-coumaroylcatalpol, premnosidic acid, 10-O-trans-p-coumaroyl-6-O-α-l-rhamnopyranosyl catalpol and a new dimeric lignin were responsible for the antioxidant effect of the stem bark (Yadav et al., 2011). Similarly the leaves and roots of *P. serratifolia* have also been demonstrated to possess antioxidant effect (Gokani et al., 2011; Selvam et al., 2012). To date a number of phytochemical studies on the leaves (Otsuka et al., 1993), stem bark (Yuasa et al., 1993) and root bark (Yadav et al., 2010) of the plant are reported but the root wood that plays major role in the Ayurvedic medicine has not yet been studied. We herewith report the identification of the antioxidant principles of the root wood tissues.

**Materials and Methods**

**General methodology**

$^1$H NMR, $^{13}$C NMR and 2D-NMR (COSY, NOESY, HSQC and HMBC) spectra were obtained on a JEOL 500 MHz instrument. Homonuclear $^1$H connectivities were determined by using the COSY experiment. One bond $^1$H–$^{13}$C connectivities were determined with HSQC while two- and three-bond $^1$H–$^{13}$C connectivities were determined by HMBC experiments. Chemical shifts were reported in $\delta$ (ppm) using the solvent standard and coupling constants ($J$) were measured in Hz. A Waters Synapt G2 TOF mass spectrometer (Waters, UK) with an electrospray ionisation probe was used to acquire data over a mass range of 50-800 u. A lock spray correction was applied to each acquired data set using Leu-Enk. The mass spectrometer was coupled with an Acquity UPLC system comprising of Acquity UPLC BEH C18 1.7 μm 2.1x50 mm column and Waters TUV at 220nm detector. Samples dissolved in methanol (0.1 mg/ml) were injected into the LC-mass spectrometer operating in a positive ion mode.

**Plant material**

The roots of *P. serratifolia* growing for several years in our experimental field (Kurunya Lane, Manganam Kara, Kalathilpady, Kottayam-10, India) were used. The location of the plant specimen source lies between 09° 34.978’ north latitude and 076° 33.146’ east longitudes. A voucher specimen numbered 2517 was kept in the CMS Herbarium, C. M. S. College, Kottayam.

**Processing and extraction of the plant material**

The bark and woody tissues of the plant were separated, air dried and grounded. The root bark (190 g) and root wood (330 g) were extracted twice by soaking in methanol (2.5 l) for two weeks. Removal of the solvent under reduced pressure yielded 20 and 19 g yield for the root bark and root wood tissues respectively.
**Isolation of acteoside from *P. serratifolia* root wood**

The methanol extract of the root wood which displayed antioxidant activity when assessed by DPPH (1, 1-diphenyl-2-picryl hydrazyl; Sigma-Aldrich, UK) radical scavenging assay was taken for isolation of the active constituents. RediSep Rf flash silica column (24g; Presearch, Hampshire, UK) attached to a Teledyne Isco flash chromatography system was used for fractionation and isolation of acteoside. Briefly, a linear gradient of methanol starting from 0% to 40% over a period of 30 minutes were established. The chromatogram was monitored by observing absorbance at dual wavelengths: 214 and 254 nm. Four major fractions were obtained: I (0-8 min), II (10-17 min), III (17-25 min) and IV (25-30 min). Fractions II and III that showed antioxidant activity were further purified using the same system to provide acteoside in the yield of 30% (w/w versus the crude extract).

**HPLC analysis**

An Agilent 1200 series gradient HPLC system composed of degasser (G1322A), quaternary pump (G1322A), auto sampler (G1329A), thermostat column compartment (G1316A) maintained at 25 °C and a diode array detector (G1315D) was used. Plant extracts dissolved in methanol (1 mg/ml) were injected (20 µL) onto a reverse phase column (Agilent – Eclipse Plus C18, 5 µm, 4.9 x 150 mm). The mobile phase was a mixture of water (A) and methanol (B). The composition of the mobile phase at a flow rate of 1 ml/min was rising from 10% to 90% B over a period of 50 minutes.

**DPPH radical scavenging activity**

The antioxidant activity of test samples was measured by using our established microtitre-based DPPH assay (Habtemariam, 2007). Briefly, DPPH solution (0.1 mM, in methanol) was incubated with varying concentrations of test compounds for 20 min at room temperature and the absorbance of the resulting solution was read at 550 nm against a blank using Multiscan EX Reader (Thermo Labsystems, Altrincham, UK).

**Results and Discussion**

The radical scavenging activity of the crude methanolic extracts of *P. serratifolia* root tissues was assessed by using the DPPH bleaching assay as described previously (Habtemariam, 2007). The typical concentration-dependent effect shown in Figure 1 was in agreement with previous studies that revealed antioxidant activity for the leaves and stem bark of *P. serratifolia* (Gokani et al., 2011; Selvam et al., 2012; Yadav et al. (2011). When the root bark and root woody tissues were separately assessed for their antioxidant activity, we observed a super-imposable activity profile (Figure 1). The IC$_{50}$ values obtained from four independent experiments were 72.5 ± 6.3 and 73.8 ± 2.4 µg/ml for bark and woody tissues of the root, respectively. This statistically insignificant difference ($p > 0.05$, unpaired t-test), suggests that both the root wood and root barks must be considered for establishing the scientific evidence of the reputed medicinal uses of *P. serratifolia* roots. Since the chemistry of the root bark has been studied in recent years, emphasis was given in the present study on the root woody tissues that is obtained in similar yield as the root bark.
HPLC analysis of the antioxidant woody tissue extract revealed one prominent peak (retention time of 23.9 min) within the 50 min analysis time (Figure 2). It appears from this figure that the extract was made of one major constituent in an incredibly pure form instead of being complex mixture as expected in crude extracts. NMR analysis, however, revealed that the extract is a crude mixture suggesting that there are lots of compounds that were not picked up by the UV detection system. Further purification was done by a fast Combiflash ISCO chromatography system that allows the processing of large scale separations. Repetitive purification using this system afforded the antioxidant principle that is shown as a major HPLC peak in the HPLC chromatogram (Figure 2). Antioxidant activity study of the isolated
active compound revealed a better activity profile than the crude extracts (Figure 2). The IC\textsubscript{50} value of the compound was also 18.3 ± 3.7 \(\mu\)g/ml and was about 4-times more potent the crude extracts.

The \(^1\)H and \(^{13}\)C NMR data clearly indicated the presence of a caffeic acid, phenyl ethanol and two sugar moieties (Table 1). Direct H-C connectivities were established by HSQC studies while \(^1\)H NMR signals were identified through the use of COSY NMR studies. The observed NMR data were in general in good agreement with those previously published for acteoside (Li et al., 2005; Otsuka et al., 1993). Further evidence corroborating the sites of linkage of the different structural moieties as shown in Figure 3 came from the HMBC studies (Table 1). The key feature of this studies (Table 1) were the observation of the expected \(^3\)J coupling (Table 1) as depicted in the structure of acteoside. In the MS study, the molecular ion appeared as a sodium adduct of acteoside ([M+Na\(^+\)]\(^+)\) at m/z 647.1958. The classical fragmentation at m/z 535, 461, 325 and 163 were further consistent with those previously reported for acteoside (Li et al., 2005). On the basis these spectroscopic data, the antioxidant principle isolated from the root wood tissues was identified as acteoside (Figure 3). The compound was previously isolated from the leaves of \textit{Premna corymbosa} that is considered to be the same (synonym) as \textit{P. serratifolia}.

Table 1. \(^1\)H, \(^{13}\)C and HMBC NMR data for acteoside. All experiments were run in CD3OD–δ values in ppm and coupling constant (J) in parenthesis are shown.

<table>
<thead>
<tr>
<th>Moiety</th>
<th>Position</th>
<th>(^1)H NMR (δ\textsubscript{H})</th>
<th>(^{13})C NMR (δ\textsubscript{C})</th>
<th>Major HMBC Correlations ((^2)J, (^3)J)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl ethanol</td>
<td>1</td>
<td>-</td>
<td>131.4</td>
<td>C-1, C-4,</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.61 d (2.2)</td>
<td>117.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>146.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
<td>144.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.60 d (8.1)</td>
<td>116.1</td>
<td>C-1, C-3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6.48 dd (8.1, 2.2)</td>
<td>121.3</td>
<td>C-2, C-4</td>
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<tr>
<td></td>
<td>7</td>
<td>2.78 m</td>
<td>36.5</td>
<td>C-1, C-2, C-6, C-8</td>
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<tr>
<td></td>
<td>8</td>
<td>3.7 and 4.04 m</td>
<td>72.3</td>
<td>C-1*</td>
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<td>-</td>
<td>127.6</td>
<td>C-4', C-6'</td>
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<td></td>
<td>2'</td>
<td>6.98 d (2.2)</td>
<td>115.2</td>
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<td></td>
<td>3'</td>
<td>-</td>
<td>146.8</td>
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<tr>
<td></td>
<td>4'</td>
<td>-</td>
<td>149.8</td>
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<tr>
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<td>5'</td>
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<td>C-1', C-3'</td>
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<tr>
<td></td>
<td>6'</td>
<td>6.85 dd (8.3, 2.2)</td>
<td>123.2</td>
<td>C-2', C-4'</td>
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<td>7'</td>
<td>7.50 d (15.8)</td>
<td>148.0</td>
<td>C-6'</td>
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<td>8'</td>
<td>6.19 d (15.8)</td>
<td>114.7</td>
<td>C-9*</td>
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<tr>
<td>Glucose</td>
<td>9''</td>
<td>-</td>
<td>168.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1''</td>
<td>4.49 d (7.8)</td>
<td>104.2</td>
<td>C-8</td>
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<td>3.84 t (9.0)</td>
<td>81.7</td>
<td>C-1''', C-2'', C-5''</td>
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<td></td>
<td>4''</td>
<td>4.90 t (9.3)</td>
<td>70.4</td>
<td>C-9'', C-3''', C-2''</td>
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<tr>
<td></td>
<td>5''</td>
<td>3.52 m</td>
<td>76.0</td>
<td></td>
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<tr>
<td></td>
<td>6''</td>
<td>3.63 and 3.52 br d (11.5)</td>
<td>62.3</td>
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<tr>
<td></td>
<td>1'''</td>
<td>5.18 d (1.6)</td>
<td>103.3</td>
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<td>2'''</td>
<td>3.91 dd (3.1, 1.8)</td>
<td>72.3</td>
<td>C-3'''', C-2/C-3, C-5'''</td>
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<td>3.57 br d (7.9)</td>
<td>72.0</td>
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<td></td>
<td>6'''</td>
<td>0.99 d (6.2)</td>
<td>18.4</td>
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*\(^3\)J connectivities showing the linkage of sub-structural moieties are shown in bold red fonts.
Figure 3. Structure of acteoside (verbacoside).

Acteoside isolated from several plants have been shown to possess potent antioxidant effects in a variety of in vitro assay models (Zaabat et al., 2011; Kim et al., 2009). Acteoside by its own or in crude plant extracts have also shown to display in vitro and in vivo anti-inflammatory (Akdemir et al., 2011; Kim et al., 2009; Korkina et al., 2007; Schapova et al., 1998; Speranza et al., 2010), wound healing (Akdemir et al., 2011; Korkina et al., 2007), antinociceptive (Akdemir et al., 2011; Backhouse et al., 2008) and hepatoprotective effects against carbon-tetrachloride (Lee et al., 2004) and D-Galactosamine/lipopolysaccharide (Xiong et al., 1999). Protection of human neuroblastoma SH-SY5Y cells against β-amyloid-induced cell injury (Wang et al., 2009), 1-methyl-4-phenylpyridinium ion-induced neurotoxicity (Sheng et al., 2002) and rat cortical cells from glutamate-induced excitotoxicity (Koo et al., 2006) are among the various neuroprotective effects of acteoside reported so far. Upregulation of heme oxygenase-1 by acteoside through ERK and PI3 K/Akt pathway has been shown to confer neuroprotection against beta-amyloid-induced neurotoxicity (Wang et al., 2012). The potential anticancer activity of acteoside is also evident from studies showing direct inhibition of human promyelocytic HL-60 leukemia cell proliferation (Lee et al., 2007) and PMA-induced invasion and migration of human fibrosarcoma cells (Hwang et al., 2011). Antimicrobial effects including activity against multiple-drug-resistant strains of Staphylococcus aureus (Nazemiyeh et al., 2008) have also been reported for acteoside. In view of acteoside’s wide range of pharmacological effects, the finding of it being a major constituent of the root woody tissues could explain the reported medicinal value and pharmacological effects of the plant.
The present finding was also in good agreement with our previous reports that showed *Premna species* as good sources of pharmacologically active natural products (Habtemariam, 2003; 1995; Habtemariam et al., 1993; 1992; 1990). The identification of the active principles from these plants would allow the standardisation and modernisation of crude drug preparations like those comprising *P. serratifolia* in the Ayurvedic medicine.

In conclusion, the roots of *P. serratifolia* are extensively used in the Ayurvedic system of medicine for treating various disease conditions. As with the other plant parts, the roots have been shown to display antioxidant activities but the active principles have so far not identified. Our phytochemical analysis using antioxidant assay as a guide has resulted in the identification of acteoside as the active principle of the root woody tissues. This compound could account for most of the pharmacological activities reported for *P. serratifolia* roots.

**Conflict of interest**

The authors declare that there is no conflict of interest.

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**References**


