# ISOLATION AND IDENTIFICATION OF PHENOLIC COMPOUNDS FROM LEAF EXTRACTS OF *COCHLOSPERMUM RELIGIOSUM* (L.) ALSTON BY ELECTROSPRAY IONIZATION MASS SPECTROMETRY

*Sasikala A, Linga Rao M* and *Savithramma N*

Department of Botany, Sri Venkateswara University, Tirupati - 517502, Andhra Pradesh, India.

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<th>ARTICLE INFO</th>
<th>ABSTRACT</th>
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<td><strong>Article history</strong></td>
<td>The present study intended to isolate phenolic compounds from the leaf of <em>Cochlospermum religiosum</em> by using 70% acetone and poly vinyl pyrrolidane; and characterized by UV-visible spectrometry, high performance liquid chromatography/electrospray ionization mass spectrometry. The results showed that totally 87 phenolic compounds were obtained at both positive and negative ion modes of LCMS. Among the isolated phenols, 11 phenolic compounds have been identified based on their retention time and m/z values namely, Gallic acid, 2,3-Dihydroxybenzoic acid, Ascorbic acid, Quercetin, Cynarine, 1,2,3,4,6-Pentagalloyl glucose, Syringaldehyde, Thymol, Ellagic acid, Isorhamnetin and Benzoic acid. This study illustrates the rich array of phenolic compounds of leaves of <em>Cochlospermum religiosum</em> could be utility as health beneficial bioactive compounds.</td>
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| **Keywords** | **Cochlospermum religiosum**, leaves, phenolic compounds, liquid chromatography, Electro Spray Ionization mass spectrometry. |

**Corresponding author**

A. Sasikala
Research Scholar
Department of Botany,
Sri Venkateswara University,
Tirupati – 517 502.
Andhra Pradesh, INDIA.
asasikala.jl@gmail.com

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INTRODUCTION

*Cochlospermum religiosum* (L) Alston is a sparsely branched small tree, belonging to the family Cochlospermaceae. It is commonly called as Yellow Silk Cotton, Buttercup Tree and Torchwood Tree because of large, bright golden yellow flowers and seeds covered with silky hairs. *C. religiosum* stem bark and root powder is traditionally used for fertility and ash of fruit mixed with coconut is used for the treatment of scabies [1]. The gum of *C. religiosum* is also found to be an ingredient of unani medicine Qurs-e-Sartaan Kafoori which is used for Styptic, Antipyretic, Phthisis, Tuberculosis, Hectic fever and Qurs-e-Suzak Cicatrizant, Diuretic, Gonorrhea. These formulations were found to possess good antibacterial and antifungal activity [2]. Sasikala and Savithramma [3] studied the antimicrobial activity of biological synthesis of silver nanoparticles from leaves of *C. religiosum* and also studied the quantitative and quantification of phytochemicals [4, 5], *in vitro* propagation [6] and histochemicals [7].

<table>
<thead>
<tr>
<th>S. No</th>
<th>[M-H] VALUE</th>
<th>Name of the compound</th>
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<tbody>
<tr>
<td>1</td>
<td>169</td>
<td>Gallic acid</td>
</tr>
<tr>
<td>2</td>
<td>315</td>
<td>2,3-Dihydroxybenzoic acid</td>
</tr>
<tr>
<td>3</td>
<td>175</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>4</td>
<td>301</td>
<td>Quercetin</td>
</tr>
<tr>
<td>5</td>
<td>258</td>
<td>Cynarine</td>
</tr>
<tr>
<td>6</td>
<td>939</td>
<td>1,2,3,4,6-Pentagalloyl glucose</td>
</tr>
<tr>
<td>7</td>
<td>183</td>
<td>Syringaldehyde</td>
</tr>
<tr>
<td>8</td>
<td>149</td>
<td>Thymol</td>
</tr>
<tr>
<td>9</td>
<td>301</td>
<td>Ellagic acid</td>
</tr>
<tr>
<td>10</td>
<td>315</td>
<td>Isorhamnetin</td>
</tr>
<tr>
<td>11</td>
<td>121</td>
<td>Benzoic acid</td>
</tr>
</tbody>
</table>

Plants are sessile organisms that cannot evade their environment and have thus evolved all sorts of plastic mechanisms to deal with a wide range of potential threats. Among those mechanisms, the expansion of secondary metabolites with defence, communication and protection roles is now considered to be particularly important. The range of secondary metabolites including phenols, amines, indoles, alkaloids and sulphonates may act as reductant substrates of peroxidases [8]. Phenolic compounds are one of the most diverse groups of phytochemicals that are universally distributed in fruits, vegetable and herbs. Approximately 8000 phenolic compounds have been isolated from natural resources [9]. Polyphenols in nature generally occur as conjugates of sugar, usually o-glycosides. Phenolic acids contain two distinctive carbon frame works, the hydroxyl cinnamic and hydroxyl benzoic structures [10]. Although the basic skeleton remains the same, the numbers and positions of the hydroxyl groups on the aromatic ring make the difference and establish the variety [11]. Many activities have been reported for most of the phenolic compounds from plants. They act as anti-oxidant, anti-inflammatory, anti-viral and anti carcinogenic agents [12].

MATERIAL AND METHODS

Extraction of polyphenols from leaf

The fully matured healthy leaves of *Cochlospermum religiosum* were collected from Tirumala forest area of Chittoor District of Andhra Pradesh, India during July, 2012. The materials were washed thoroughly and shade dried. 30 g of leaf powder reduced in a mortar and consequently extracted with 500 mL of dichloromethane by ultra-sonication for about 30 min and shaken by vortex for 30 min to remove hydrophilic compounds. Dilapidated powder were extracted with 500 mL of acetone/water (70:30, v/v) by sonication for 15 min and shaking for more 15 min to extract polyphenols. 150 mL of each leaf water extract (pH adjusted to 4.0) was mixed with 5 g of PVPP (30 mg/mL) for 15 min of shaking for adsorption of phenolic compounds to PVPP (Sample-1). The remaining PVPP was re-extracted twice again with 200 mL of fresh extraction solvent for the same period of time that was used before. The combined extracts were evaporated at room temperature by rotary evaporation to remove the organic solvent (acetone) (Sample-2).
HPLC-ESI-MS/MS analysis

The qualitative study of the phenolic compounds in all samples was performed by HPLC coupled on-line with electrospray ionization (ESI) mass spectrometry. The HPLC system (Agilent 1100 series) consisted of a low-pressure quaternary pump (Agilent 1100 series) and an auto-sampler. A quadropole ion trap mass spectrometer (Agilent 1100) equipped with an ESI source in the positive and negative ion mode and Xcalibur software Version 1.4 (Finnigan) were used for data acquisition and processing.
RESULTS AND DISCUSSION

In this study over 87 (Positive mode-71 and Negative mode-16) phenolic compounds were extracted from lyophilized leaf samples of *Cochlospermum religiosum*. Among these 11 phenolic compounds were identified by comparing LCMS spectral data with those of literature data. The identification of these compounds was summarized in Table-1. Structures of various isolated phenolic compounds are presented in Fig-5.
Liquid chromatography, electrospray ionization – Tandem mass spectrometry (LC-ESI-MS) has been given MS fragmentation data for structural characterization of the extracted phenolic compounds. Identification has been carried out based on their pseudomolecular [M-H] ions and m/z values of mass chromatography (Fig-1-4). ESI operated in negative mode, which is known as a soft and highly efficient ionization method, proved to be an excellent tool for the identification of flavonoid glycosides by providing information on the glycoside molecular masses due to their prominent [M-H]\(^+\) ions and fragmentation products of the aglycone arising from Retro-Diels-Alder reactions [13-15]. ESI-MS parameters were optimized for the components and the solvent system, respectively to maximum ionization efficiency fragmentation of phenolic acid yield product ions more efficiently at lower collision energies. While flavonols and particularly their aglycones needed higher collision energies to obtain diagnostically relevant product ions [16].

Thymol was identified as it showed identical LC MS characteristics as that of the standards m/z 149. It will produce the fragments at m/z 131 and m/z 120 by the loss of water and an ethyl (C\(_2\)H\(_5\)-CH\(_2\)) group. Similar results have been obtained from Lamiaceae species by Hossain [17]. Among the eluted phenolic compounds, benzoic acid (m/z 121) is also identified. These are also reported from Oak barrels by Regalado et al., [18].

The presence of free ellagic acid was confirmed by its retention time and MS data with m/z 301. This fragment showed in higher mass region with a relatively high intensity. The molecular ion at m/z 447 can yields fragments at m/z 315 and m/z 301. This peak could be a methyl ellagic acid pentose conjugate as described by Mullen et al. [19] Zhang et al. [20] Soong and Barlow [21].

Gallic acid was identified based on the m/z value 169. Gallic acid exist either in the free form or bound as gallo tannins, these hydrolysable tannins are present in a rich variety of plants and are present in tea, red wine, fruits beverages and various plant material [22] and is generally regarded as safe food additive functioning as an antioxidant in some countries including Japan [21]. Gallic acid is known to have anti-inflammatory anti mutagenic, anticancer and antioxidant activity [23]. Pentagalloyl glucose was observed at anion m/z 939. The same was reported by Soong and Barlow [21].

Several studies have indicated a high degree of compartmentation of phenolic compounds and of the enzymes involved in their biosynthesis. Phenolics usually accumulate in the central vacuoles of guard cells and epidermal cells as well as subepidermal cells of leaves and shoots. Furthermore, some phenolics are found covalently linked to plant cell wall, others occur in waxes or on the external surfaces of plant organs. Some findings suggest that a deposition of flavonoids in nuclei of certain tree species. It has been suggested that a flavonoid-DNA complex provides a mutual protection against oxidative damage [24-25].

Leaves of Cochlospermum religiosum is rich source of phenolic compounds. Moreover, these phenols could be used as natural antioxidants substituting the synthetic antioxidants in food, cosmetic and pharmaceutical industries.

**REFERENCES**


