IDENTIFICATION OF PHYTOCHEMICAL CONSTITUENTS FROM
TAMILNADIA ULIGINOSA (RETZ.) TIRVENG. & SASTRE
(RUBIACEAE) FRUITS USING HPLC ANALYSIS.

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ABSTRACT
Tamilnadia uliginosa (Retz.) Tirveng and sastre (Rubiaceae), known as Divine Jasmine, is one of the renowned medicinal plants used to treat large number of human ailments mentioned in Ayurveda, Siddha and Unani. The fruits are edible and eaten as vegetable. It is also diuretic, purgative, aphrodisiac and useful for the treatment of burning sensation, piles, sexual weakness and dysentery. Rural people in Wayanad district of Kerala are using fresh fruit of T. uliginosa for the treatment of dysentery and diarrhea, and also used for culinary purposes. But only stray reports on the phytochemical constituents of the fruits. Preliminary phytochemical screening of methanolic extract of fruit revealed the presence of phenolic compounds in high amounts. Hence an attempt was made to identify its phytoconstituents of T.uliginosa fruit through HPLC analysis. The analysis revealed the presence of 15 compounds, most of them are phenolic compounds. Phenolic compounds having potential anticancer and antioxidant properties, hence the result clearly shows the potential phytotherapeutic value of this species.

KEY WORDS: Tamilnadia uliginosa, phytochemical screening, HPLC, phenolic compounds.

INTRODUCTION
Tamilnadia uliginosa (Retz.) Tirveng and sastre, belongs to the family Rubiaceae, is a small tree distributed in Indian subcontinent (Bangladesh, India, Sri Lenka) and Indochina (Thailand, Vietnam). In Kerala it is usually growing in the moist deciduous forest ranging
from Wayanad to Thiruvananthapuram (Agrawal and Sing 1999). But in this present study, it was located only in Wayanadu district, where it seen as wild. *T. uliginosa* is a deciduous, thorny, rigid shrub. The raw fruit extract is used against diarrhea and dysentery. Fruit pulp is applied for curing boils. The fruits are rich source of carbohydrate and possess insecticidal properties. Ripe fruit contains triterpenoid glycosides. It cures abscess, ulcers, inflammations, wounds, tumours and skin diseases (Nadkarni et al., 1976). The present study was carried out to analyse the identification of phytoconstituents present in *T. uliginosa* fruit. The study includes HPLC analysis of fruit extracts by successive solvent extraction for detailed analysis. The present work is designed to explore the phytochemical constituents of *T. uliginosa* fruit, which is responsible for its various pharmacological properties using HPLC analysis.

**MATERIALS AND METHODS**

Fresh fruits of *Tamilnadia uliginosa* were collected from Wayanad District. These fruits were washed well using tap water followed by distilled water, cut into pieces and dried in shade for a period of 20-25 days, at an ambient temperature of 25°C. The dried samples were ground initially in a mortar and pestle followed by a mixer grinder, to obtain the fine powder.

**HPLC Analysis:** The HPLC analysis was performed using a LC-solution, Shimadzu TM, MAO 1527, USA with LC-UV-100 UV detector, A CAPCELL (C- 4.0 mm), type MG 5-20 μm, number×18 column RP (18.5μm, 250 AKAD/05245). HPLC grade solvents were obtained from S.D fine chemical Ltd. Mumbai (Hao Chen et al., 2001). The mobile phase consisted of solvent mixtures [Methanol: Water (80:20)] and [ACN: Water (80:20)]. Isocratic elution (0-12min) was done with a flow rate of 1.0 ml/min at a column temperature of 30°C. The injection volume was 20 μl, and UV detection was effected at 276 nm and 273 nm.

**RESULTS**

**HPLC analysis:** HPLC profiles of 15 phenolic compounds having different elution times and area could be obtained when each compound was analyzed individually using the mobile gradient phase consisting of methanol and 1% acetic acid in water for 25 min running time (Fig.1 -2 and Table 1). The chromatographic fingerprints of these phenolic compounds when mixed together exhibited the same elution sequence, the combined chromatogram resembling a synchronized assembly of the individual HPLC profiles with the
individual peak area and times mentioned here. Highest peak area is obtained for luteolin followed by quercetin. These are coming under the category of phenolic compounds.

![Area Percentage](image1)

**Fig 1. Standard graph**

![HPLC chromatogram](image2)

**Fig 2. HPLC chromatogram of *T. uliginosa* fruits**

**Table 1. HPLC analysis data of methanolic fruit extract of *T. uliginosa***

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>R.T</th>
<th>AREA</th>
<th>AREA %</th>
</tr>
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<tbody>
<tr>
<td>VITEXIN</td>
<td>4.03</td>
<td>406756</td>
<td>6.344</td>
</tr>
<tr>
<td>RUTIN</td>
<td>6.5</td>
<td>178981</td>
<td>2.567</td>
</tr>
<tr>
<td>QUERCETIN-3-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GALACTOSIDE</td>
<td>9.24</td>
<td>78965</td>
<td>1.378</td>
</tr>
<tr>
<td>LEUTEOLIN-7-GLYCOSIDE</td>
<td>10.22</td>
<td>119018</td>
<td>3.068</td>
</tr>
<tr>
<td>QUERCETIN-3-GLYCOSIDE</td>
<td>11.68</td>
<td>75645</td>
<td>1.289</td>
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<tr>
<td>QUERCETIN</td>
<td>13.46</td>
<td>644887</td>
<td>11.061</td>
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<tr>
<td>MYRICETIN</td>
<td>14.89</td>
<td>227861</td>
<td>3.567</td>
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<tr>
<td>LUTEOLIN</td>
<td>18.09</td>
<td>775645</td>
<td>13.265</td>
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<tr>
<td>APIGENIN</td>
<td>19.56</td>
<td>406754</td>
<td>6.923</td>
</tr>
<tr>
<td>KAEMPFEROL</td>
<td>21.7</td>
<td>396754</td>
<td>6.897</td>
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<td>HYPEROSIDE</td>
<td>24.89</td>
<td>270565</td>
<td>4.57</td>
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<tr>
<td>ISORHAMNETIN</td>
<td>27.25</td>
<td>14087</td>
<td>0.245</td>
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<tr>
<td>CAFFEIC ACID</td>
<td>29.91</td>
<td>497865</td>
<td>8.565</td>
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<tr>
<td>FERULIC ACID</td>
<td>31.42</td>
<td>44565</td>
<td>0.809</td>
</tr>
<tr>
<td>NONACOSANE</td>
<td>36.62</td>
<td>445645</td>
<td>7.556</td>
</tr>
</tbody>
</table>

**DISCUSSION**

High Performance Liquid Chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products. HPLC analysis can be used for classification
of herbs based upon secondary metabolites. Extracts of the plant parts at optimum conditions were analyzed by HPLC for quantifying bioactive polyphenolic compounds, such as phenolic acids and flavonoids, the important constituents in many plants. Their identification and quantification can give vital information relating to antioxidant function, food quality, and potential health benefits. Phenolic acids are attractive as they are known to act as potentially protective factors against cancer and heart diseases (Hermann et al., 1999; Aruoma, 1999).

Quercetin is a plant derived flavonoid used as a nutritional supplement. It is found in fruits and vegetables. Flavonoids (collectively known as Vitamin-P) and citrine are a class of secondary metabolites. Almost all universal pigments of plants are flavonoids (Pepeljnjak et al., 2005). Vitexin may increase antioxidant enzyme activity, has potent and broad antitumor efficacy in ectopic growth of breast, prostate, liver and cervical cancer cells (Lee et al., 2005). Rutin has a broad range of physiological activities such as anti-inflammatory, antitumour and antibacterial activities. Ferulic acids are transisomers; they are esterified at their carboxyl groups to polysaccharides (Hartley, 1973). Nonacosane occurs naturally and has been identified within several essential oils. It can be prepared synthetically (Brei et al., 2004).

High Performance Liquid Chromatography has proved to be the method of choice for the separation of a variety of flavonoids present in the fruit sample. In the present study, we have found that the medicinal properties of T. uliginosa fruits may be due to the presence of above mentioned compounds. The HPLC chromatogram will help as standard chromatogram in future studies. These chromatograms can be used as finger prints for the compounds obtained from this plant.

CONCLUSION

The method was validated and found to be simple, sensitive, and accurate and precise. It can also be used for further impurity profiling of the drug. Although the method could effectively separate the drugs from its degradation products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulation.

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REFERENCES