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Short Communication

Detection of Arctigenin in *Ipomoea cairica* L. leaves: A potential drug for Japanese encephalitis

Deepa Srivastava
Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh, India
drdeepasrivastava@yahoo.com

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Abstract

Ipomoea cairica L. is a perennial herb which grows in unstable sites, such as waste-ground and roadsides in urban areas. It has been reported for several medicinal properties. Literature survey revealed the presence of phenylpropanoid compounds in this plant. One of such compound is Arctigenin. Arctigenin, is a Phenylpropanoid dibenzylbutyrolactone lignan with antinflammatory and antioxidant activities. Recent report suggests that arctigenin inhibits neuronal apoptosis and can reduce the severity of Japanese encephalitis. The presence work confirms the presence of arctigenin in cultivated plant of Ipomoea cairica leaves extract. The dried leaves sample was extracted through column chromatography with ethanol. The extracted samples were subjected to thin layer chromatography (TLC) taking Benzene: Ethyle acetate (9:1 ratio) and high performance liquid chromatography (HPLC) in methanol. Identification of the isolated compounds was done with reference standard using TLC and HPLC. The present study confirms the presence of arctigenin in leaves extract of Ipomoea cairia leaves.

Keywords: *Ipomoea cairica*, Arctigenin, HPLC, TLC, Japanese encephalitis, column chromatography.

Introduction

Ipomoea cairica, commonly known as rail road wine is an environmental weed, found in waste land areas. It has been reported as a promising medicinal plant due to presence of various phytochemicals such as flavonoids, alkaloids, Tannins, saponins, phenols, amino acids, glycosides, anthraquinones, steroids and lignans. The literature provides numerous records on their antifungal, anti-inflammatory, antioxidant, hepato-protective, antitumor, antimicrobial, anti-aging and hypoglycemic properties[1-3].

Arctigenin (Figure-1) is a naturally occurring phenyl propanoid dibenzyl butyrolactone lignan reported to be present in *Arctium lappa*, *Bardane fructus*, *Cincus benedictus*, *Frosythia intermedia*, *Merremia gemella, Ipomoea cairica*, *Saussurea medusa* and *Torreya nucifera*. It is extracted from *Arctium lappa* and *Arcitium tomentosum* [4,5]. It possess a range of pharmacological activities including anti-inflammatory, antioxidant, anti-cancerous, anti-proliferative and antiviral activity[6-8]. It has shown gastroprotective effect and found to be protective for endole induced neurotoxicity in PC 12 cells[9,10]. It is also found useful in Alzheimer's disease in vitro study[11]. It has been reported neuroprotective against Japanese encephalitis in a mouse model[12,13]. Very little work has been done on content of arctigenin in different part of *Ipomoea cairica.* This work will confirm the presence of Arctigenin compound in *Ipomoea cairica*, in leaf samples.

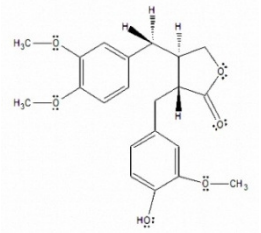

Figure-1: Structure of arctigenin.

Materials and methods

Preparation of Sample: *Ipomoea cairica* leaves were collected from planted material in University Campus of D.D.U. Gorakhpur University, Gorakhpur. The collected leaves were washed properly with distilled water and shade dried for 15 days. The dried leaves were crushed using a mixture grinder and stored in air tight containers for further studies.

Preparation of Standard Solution: Arctigenin was purchased from Cayman Chemical Company, USA products no 14913. A standard solution was prepared by dissolving 0.49 mg/mL of arctigenin, in ethanol (HPLC grade purchased from Merk).

Column Chromatography: The 100 mg of dried powdered leaves were soaked overnight in ethanol (300ml) then filtered and subjected to column chromatography. The mobile phase was ethanol. The samples were collected in 30 different

fractions in collection tube. Each fraction was tested for presence of Arctigenin with the help of TLC.

Thin Layer Chromatography (TLC): TLC silica gel 60 F_{254} plates were taken from Merck. The standard solution of arctigenin and leaf extract samples collected from Column chromatography was subjected to TLC plates by capillary tube. The solvent system taken was Benzene: Ethyle acetate (9:1). The chromatograph was developed in presence of Iodine crystal.

High Performance Liquid Chromatography (HPLC): The sample confirmed by TLC for the presence of arctigenin was analysed by HPLC. Kromasil C18 column (column size-250x4.6mm, 5µm) was used for HPLC analysis. Detection wavelength was kept 220nm and methanol: water 60:40 ratios were taken as mobile phase. The flow rate was 5.0ml/min. The run time was 60min and 10µL was the specimen handing quantity. All separations were performed at room temperature.

Results and discussion

Column Chromatography Analysis: The extracted samples obtained from column chromatography were collected in 15ml collection tubes. 30 such tubes were collected (Figure-2a, b).

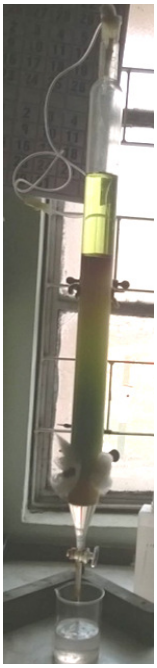

Figure-2a: Column chromatography.

Figure-2b: Sample collected from column chromatography.

Thin Layer Chromatography Analysis: The Rf value of standard solution of arctigenin and leaves sample collected from column chromatography showed the same value in TLC plates. The Rf value of pure sample (p) was 5.2 cm and Rf value of column chromatography sample was 5.1cm. Hence both represent the similar compound *i.e.* Arctigenin (Figure-3).

Figure-3: Developed TLC plate of *Ipomoea cairica* samples. P is pure compound, C sample obtained from column chromatography.

High performance thin layer chromatography Analysis (HPLC): The HPLC analysis with the above mentioned chromatographic conditions showed retention time for standard arctigenin was 4.954min (Figure-4a) while the retention time of the leaves sample obtained from column chromatography was 4.876 min (Figure-4b). The similar retention time confirms the presence of same compound *i.e.* arctigenin in the sample and standard.

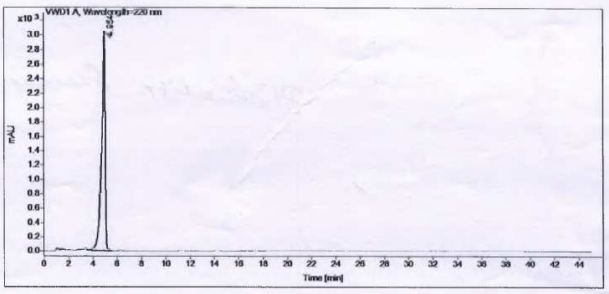

Figure-4a: The HPLC profile of standard compound of Arctigenin.

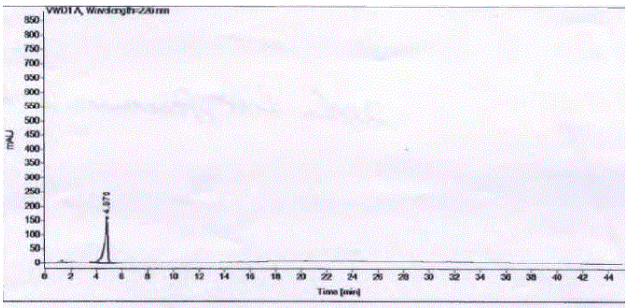

Figure-4b: The HPLC profile of compound isolated from leaves of *Ipomoea cairica* through column chromatography.

Conclusion

This study reveals that locally available unutilized weed plant *Ipomoea cairica* has great pharmaceutical potential. The developed TLC and HPLC method confirmations the presence of arctigenin in leaves sample of *Ipomoea cairica*. The short retention time and sensitivity for presence of arctigenin makes this method comfortable. Further studies are conducted to develop better methods for extraction of Arctigenin in large scale from this plant. The cost effective method for extraction of arctigenin can make this weed plant an economic asset for this area. Hence, *Ipomoea cairica,* seemed to have the potential to be used as a new therapeutic agent for endemic disease Japanese Encephalitis.

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