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Research Article

ISOLATION AND STRUCTURAL DETERMINATION OF ARCTIGENIN FROM IPOMOEA CAIRICA L. (SWEET)

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ABSTRACT

Arctigenin has many pharmacological activities with clinical significance and is mainly derived from Arctium lappa L. It has also been reported in Fructus Arctii, Bardane fructus, Merremia gemella, Ipomoea cairica, Forsythia intermedia, Saussurea nucifera and Torreya medusa. In the present investigation Ipomoea cairica L. (Sweet) is taken for isolation and structural determination of Arctigenin present in it. The leaves extract in ethanol was taken for isolation of Arctigenin; the separations were performed at preparative scale with solvent composed of Benzene: ethyle acetate (9:1) ratio. Isolation was done by silica gel column and preparative TLC. Purity checked by HPLC and TLC. The structural determination was done by Mass Spectroscopy, IR spectroscopy; NMR and elemental analysis.

INTRODUCTION

Ipomoea cairica L. (Sweet), commonly known as rail road wine is a perennial climbing herb belongs to family Convolvulaceae. Different parts of the plants possess anti-inflammatory, antioxidant, antimicrobial, anti-HIV, cytotoxic and anti-Japanese encephalitis activities [1, 2]. *Ipomoea cairica* (IC) has also shown mosquito's larvicidal activity and allelopathic activities [3, 4]. From the aerial parts of *Ipomoea cairica,* the coumarins, umbelliferone, scopoletin, and the dibenzyl- γ-butyrolactone lignans arctigenin, matairesinol and trachelogenin, were isolated along with β-sitoterol and fatty acids [5]. Arctigenin one of the phytochemicals of *Ipomoea cairica* possesses many remarkable pharmacological activities such as anti-inflammatory [6], antiviral [7, 8], anti-HIV-1[9], antitumor [10, 11] and anti-leukemia [12, 13]. It inhibits acetyl cholinesterase and scavenges nitrite [14]. It suppresses the over production of Nitric oxide [15] and also suppresses interleukin-2 (IL-2) and interferon-γ (IFN-γ) production in primary human lymphocytes [16]. Arctigenin is an effective endoplasmic reticulum stress alleviator [17]. It has shown protective effect in Parkinson disease [18]. Literature survey reveals chromatographic methods for extraction of Arctigenin from *Saussurea medusa*, *Arctium tomentosum, Forythia koreana* [19, 20, and 21]. However none of the method describes the extraction, isolation and characterization of Arctigenin from *Ipomoea cairica* in detail. In our previous work we have detected the presence of Arctigenin in *Ipomoea cairica* leaves [22]. In this paper an attempt has been made to develop a method for isolation and characterization of Arctigenin from *Ipomoea cairica*. Extraction and isolation forms an important tool for the analytical studies and hence considering the importance, the following study was undertaken.

MATERIAL AND METHODS

Materials

Arctigenin was purchased from Sigma Cayman chemical company St. Louis (USA), silica powder (G-250), TLC silica gel 60 F_{254} plates, ethanol, benzene, ethyle acetate, were from Merck Ltd Mumbai, India.

Preparation of leaves sample

Ipomoea cairica leaves were collected from Botanical garden of D.D.U.Gorakhpur University, Gorakhpur. The collected leaves were washed properly with distilled water and dried in shade. The dried leaves were crushed into powder by using and electronic mixture grinder. The powder sample was stored in air tight container for further studies.

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Extraction of Crude Sample

The powder of IC leaves was soaked in ethanol for preparation of crude sample of Arctigenin. In this experiment 100g of leaf powder soaked in 300ml ethanol (absolute) for 24 hours. The supernatant of the soaked leaves was filtered with whatman filter paper No. 1. The extract was concentrated and subjected to silica gel column chromatography.

Column Chromatography

The column chromatography for isolation for Arctigenin was carried out on silica gel (350 mesh), column (40x1cm). The solvent system used for isolation was optimized to ethanol with a rate of elution 30 drops per minute. The sample was loaded in the ratio of sample size to the amount of adsorbent (1:30). Sequentially 30 fractions of 5ml each were collected throughout the isolation process. The solvent system eluted with the fraction was completely removed by evaporation at 50^{o}C under a stream and the residue was reconstituted with 0.5 ml ethanol. After reconstruction, TLC of these fractions was carried out to find out in which fraction Arctigenin was present. The 15th fraction contained Arctigenin but none of the fraction contained exclusively Arctigenin. It was not possible to isolate exclusively Arctigenin by Column Chromatography and hence for the further purification step preparative thin layer chromatography was employed.

Preparation of Samples

Pure Arctigenin was purchased from Cayman Chemical Company, USA products no 14913. A standard solution was prepared by dissolving 0.49 mg/ml (1.3 M) of Arctigenin in ethanol. For UV identification the 0.1mg/ml sample of isolated Arctigenin in ethanol was prepared. For NMR isolated Arctigenin was dissolved in $CDCl_3$ and spectrum was recorded, for mass spectra 1g/ml solution of isolated Arctigenin in ethanol.

Thin Layer Chromatography

The quantitative analysis of Arctigenin was performed by thin layer chromatography under the following conditions; Benzene: ethyle acetate (9:1) was used as developing agent at room temperature. Iodine chamber was used as colouring agent at 30^{o}C.

High Performance Liquid Chromatography (HPLC)

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; 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 60 min and 10µL was the specimen handling quantity. All separations were performed at room temperature.

Preparative Thin Layer Chromatography (PTLC)

The structural identification is based on IR, Mass, NMR and elemental analysis of the arctigenin purified by preparative thin layer chromatography. Silica gel 60F_{254} plates were used as the stationary phase. A band of the fraction collected from the column was applied using narrow tip glass capillary. The solvent system was optimized to Benzene: ethyle acetate (9:1) which gave the best separation on silica gel plates. For detection of Arctigenin, a counter plate was run which was developed in iodine chamber. After the detection the silica gel covering the area on which Arctigenin was present was carefully scraped out and Arctigenin was recovered by eluting in ethanol. The absorbent material and solvent were homogenized on a vortex mixture centrifuged to ensure complete elution. The supernatants were collected and evaporated to obtain Arctigenin. The obtained sample was send to SAIF, CDRI for its identification from IR, NMR, Mass spectroscopy and elemental analysis.

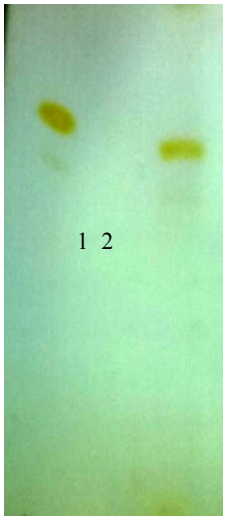

Figure 1 TLC plate of *Ipomoea cairica* samples. Lane 1 pure compound, lane 2 sample obtained from column chromatography.

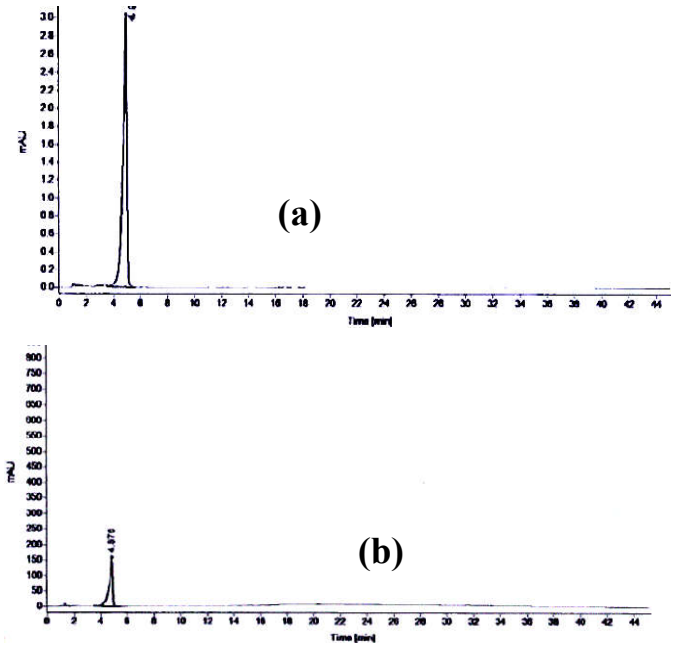

Figure 2 The HPLC profile of compound isolated from leaves of *Ipomoea cairica* through column chromatography.

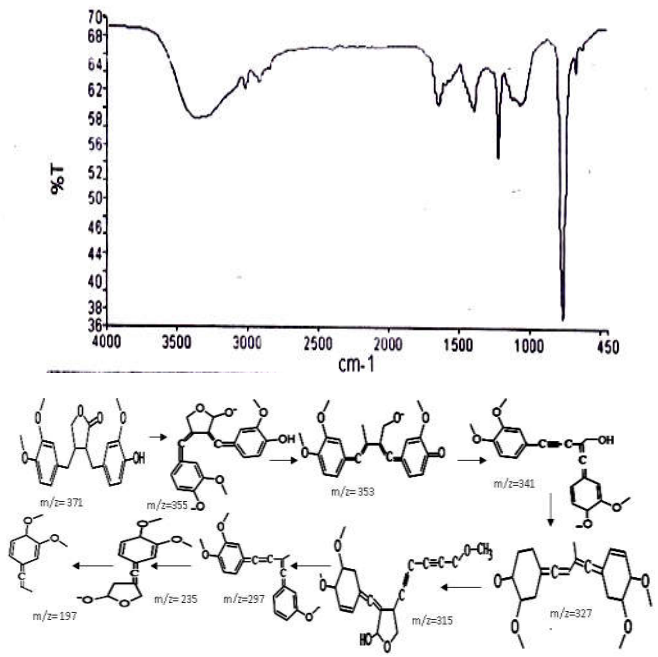

Figure 3 IR spectrum of pure compound isolated from preparative TLC of Ipomoea cairica leaf sample.

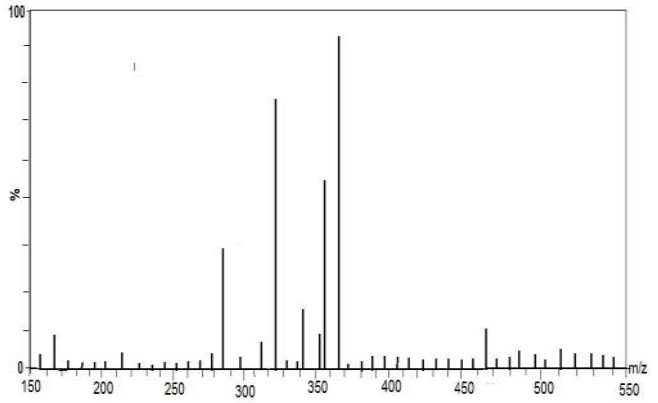

Figure 4 Mass spectrum of compound isolated from *I. carica.*

RESULT AND DISCUSSION

Extraction and Chromatographic Methods

The production of arctigenin was performed using the best extraction procedure obtained from the above experiments. The samples obtained from column chromatography were subjected to TLC. In the course of TLC analysis (shown in Fig 1) the result indicated that both arctigenin from commercial level (pure) and IC leaves shown same colour and approximately same Rf value i.e. 0.52 cm and 0.51cm respectively.

High Performance Liquid Chromatography (HPLC)

Scanning from 192nm to 400 and 220nm was selected as wavelength detection for HPLC Chromatogram. In order to achieve better chromatographic separation various linear gradients for methanol-water were investigated at flow rate of 5ml/min results are shown in Fig. 2. An HPLC chromatogram of arctigenin in standard solution is shown in Fig. 2(a) and HPLC chromatogram of arctigenin in sample solution is given in Fig 2(b). It is obvious from the figures the retention time of standard sample and IC leaves of Arctigenine are same i.e. 4.954 min and 4.876 min respectively.

FT-IR of Pure Compound Isolated from Ipomoea cairica Extract

IR spectroscopy is most commonly used in phytochemical studies as a finger printing device for comparing a natural with a synthetic reference standard. Such comparisons are very important in the complete identification of many type of plant constituents. The IR spectra peak shown in fig.-3 were displayed at 3368 cm^{-1} for stretching of –OH bond, at 3021 cm^{-1} for aromatic C-H stratching,2927 cm^{-1} for aliphatic C-H , 16-41 cm^{-1}, 1387$cm^{-1}$for C=C stretching, 1215-1063 cm^{-1} due to out of plane C-H bending of monosubstituted benzene.

LC-MS, NMR and Elemental Analysis of Compound Isolated from Ipomoea Cairica Extract

The isolated compound was confirmed by LC-MS fragmentation spectra analysis were at m/z 371the fragments peak of compound were observed at m/z 355,353,341,327,215,297,235 and 179. These fragments are characteristic peak of Arctigenin as shown in Figure- 4.

The NMR spectra of isolated compound showed peak at 2.8 ppm due to ten proton singlet of two phenyl rings. The H-NMR spectra appeared at 4.5 ppm due to CH protons . The CH_3 protons were displayed at 1.2-0.9 ppm and the OH proton peak observed at 3.1 ppm. The H-NMR spectrs appeared at 3.3ppm due to ortho H stretching (fig. not shown).

The elemental analysis results obtained from SAIF, CDRI, confirmed the presence of C, H and N in the sample. The elemental analyzer was EUROVECTOR EA 3000, SAIF No 9538, elements were N 0.022%, C 67.680%, H 7.725%. The elemental analysis of isolated product shows that the molecular formula of the product is determined as C_{21} H_{24} O_6. Thus we conclude that isolated compounds from*Ipomoea cairica* is Arctigenin.

CONCLUSION

In this study it was clearly demonstrated that locally available unutilized weed plant *Ipomoea cairica* has great pharmaceutical potential. An isolation method with ethanol was successful for preparation of arctigenin from *I. cairica*. This method is simple, rapid, sensitive and reliable. The TLC and HPLC method is suitable for quantitative analysis and quality control of *I. cairica*. Arctigenin, Isolation from local weed plant make Arctigenin available at comparative low cost hence this method makes availability of Arctigenin cost effective.

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References

1. Srivastava D, Shukla K. Ipomoea cairica: A medicinal weed with promising health benefits. I.R.R. 2015; 2(05): 687-694.

2. Srivastava D, Shukla K. Antioxidant potential of medicinal plant Ipomoea cairica (L).Sweet. I. J. D.R. 2015, 5(4):4255-4258.
3. Samuel L, Lalrotlunga, Muthukumaran RB, Gurusubramanian G, Sentikumaran N. Larvicidal activity of Ipomoea carica (L.)Sweet and Agerntina adenophora (Spreng.) Exp. Parasitol.2014; 141:112-21.
4. Srivastava D, Shahi S, Rai R, Allelopathic effect of Ipomoea cairica (L) on Noxious weed Parthenium hysterosporus Linn. Int. *J. of Curr. Res. in Life Sci*. 2015; 4(12): 489-491.
5. Lima O.de A, Filho RBH, Dibenzylbutyrolactone Lignans and Coumarins from Ipomoea cairica, Braz. Chem. Soc. 1997; 8(3): 235-238.
6. Cho MK, Jang YP, Kim YC, Kim SG. Arctigenin, a phenylpropanoid dibenzylbutyrolactone lignin, inhibits MAP kinases and AP-1 activation via potent MKK inhibition: The role in TNF-alpha inhibition. Int. Immunopharmacol. 2004; 4:1419-29.
7. Yang Z, Liu N, Huang B, Wang Y, Hu Y, Zhu Y, Effect of anti-influenza virus of Arctigenin in vivo.PubMed.gov 2005; 28: 1012-4.
8. Hayashi K, Narutaki K, Nagaoka Y, Hayashi T, Uesato S. Therapeutic effect of arctiin and arctigenin in immunocompetent and immunocompromised mice infected with influenza A virus. Biol. Pharm. Bull. 2010; 33: 1199-205.
9. Vlietinct AJ, De Bruyne T, Apers S, Pieters LA. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. Planta Med. 1998; 64:97-109.
10. Matsumoto T, Hosono-Nishiyama K, Yamada H. Antiproliferative and apoptotic effects of butyrolactone lignans from Arctium lappa on leukemic cells. Planta Med. 2006; 72-276-8.
11. Kim JY, Hwang JH, Cha MR, Yoon MY, Son ES, Tomida A, Ko B, Song SW, Shin-Ya K, Hwang Y, Park HR. Arctigenin blocks the unfolded protein response and show therapeutic antitumor activity. J. Cell Physiol. 2010; 224: 33-40.
12. Awale S, Lu Jie, Kalauni SK, Kurashima Y, Tezuka Y, Kadota S, Esumi H, Identification of Arctigenin as an Antitumor Agent having the ability to eliminate the tolerance of Cancer cells to Nutrient Starvation. Cancer Res. 2006; 66(3):1751-1757.
13. Srivastava D. Shukla K. Arctigenin: A plant lignin with tremendous potential: A review. *Int. J of Current Res.* 2017; 9 (5): 50232-50237.
14. Cai E, Han J, Yang L, Zhang W, Zhao Y, Chen Q, Guo M, He X. Novel Method of Preparation and Activity Research on Arctigenin from Fructus Arctii. Pharmacogn Mag. 2018;14 (53): 87-94
15. Zhao F, Wang Lu, Ke Liu. In vitro anti-inflammatory effect of arctigenin, a lignin from Arctium lappa L., through inhibition on iNOS pathway. *Journal of Ethnopharacology*.2009; 122: 457-462.
16. Tsai WJ, Chang CT, Wang GJ, Lee TH, Chang SF, Lu SC and Kuo YC. Arctigenin from Arctium lappa inhibits interleukin-2 and interferon gene expression in primary human lymphocytes. Chinese Medicine. 2011; 6(12): 2-8.
17. Gu Y, Sun XX, Ye JM, Li H, Yan SS, Zhang HH, Hu LH, Yuan JY, Yu Q. Arctigenin alleviates ER stress via activating AMPK. *Acta Pharmacologica Sinica*. 2012; 33: 941-952.
18. Zhang Na, Dou D, Ran X. and Kang T. Neuroprotective effect of Arctigenin against neuroinflammation and oxidative stress induced by rotenone. RSC Adv. 2018; 8:2280-2292.
19. Yu R, Liu Z, Yu R, Zhang H, Shao Y, Mei L, Tao YA. simple method for isolation and structural identification of Arctigenin from Saussurea medusa Maxim. by preparation chromatography and single crystal X-ray differentiation. *J. of Med. Plant Res*. 2011; 5(6): 979-983.
20. Zhou X, Zhang H, Liang GE, Gong H, Tian S. Determination of Arctiin and Arctigenin in Arctium Tomentosum Mill. by HPLC Method. e-J. of Chem. 2011;8(S1): S372-S376.
21. Choi YH, Kim K, Yoo KP. High performance Liquid Chromatography-Electronspary Ionization MS-MS Analysis of Forythia koreana Fruits, Leaves and Stems. Enhancement of the efficiency of extraction of Arctigenin by us of Supercritical-Fluid Extraction. Chromatographia. 2003; 57: 73-79.
22. Srivastava D. Detection of Arctigenin in Ipomoea cairica L.leaves: A potential drug for Japanese encephalitis. *Int. Res. J. of Biological Sciences*.2017; 6 (8): 47-49.

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