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High-Performance Liquid Chromatographic Method for Quantitative Estimation of an Antioxidant Principle Chlorogenic Acid in *Saussarea costus* and *Arctium lappa*

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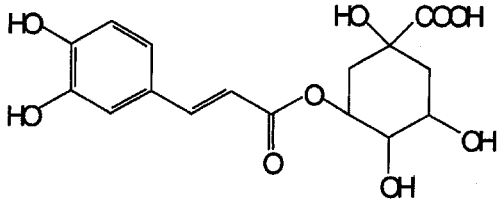
Abstract – A simple quantitative HPLC method has been developed for differentiating two plants of Asteraceae family viz. *S. costus* and *A. lappa* by using a pharmacologically active constituent chlorogenic acid and symmetry C18 column clubbed with a binary gradient using acetonitrile: 0.1% phosphoric acid and detected using a PDA at 327 nm. Quantitatively chlorogenic acid was found to be more in *A. lappa* (0.140%) than in *S. costus* (0.087%).
Keywords – *Saussarea costus*, *Arctium lappa*, HPLC, Chlorogenic acid

Introduction

Saussurea costus (Falc.) Lipsch. Syn *S. lappa* Cl. belonging to family Asteraceae is an erect robust perennial herb 1-2 m tall. It has been widely used in the indigenous system of medicine as an aphrodisiac tonic and as a valuable remedy in asthma and chest complaints. It also has strong antiseptic and disinfectant properties (Watt, 1890). Pharmacological activities carried out on *S. costus* has shown it to possess antiulcer, cholagogic effect (Yamahara *et al.*, 1985), anti-inflammatory (Cho *et al.*, 2000), inhibition of killing activity of cytotoxic T lymphocytes (Taniguchi *et al.*, 1995) and suppressing hepatitis B virus surface antigen gene expressions in human hepatoma cells (Chen *et al.*, 1995). *S. costus* has been reported to contain a number of guainolides viz. 12-methoxy dihydrodehydrocostuslactone (Dhillon *et al.*, 1987), isodehydrocostuslactone and isozaluzanin C (Kalsi *et al.*, 1984), 11,13-epoxy- isodehydrocostuslactone (Chhabra *et al.*, 1998), 4β-methoxydehydrodehydrocostuslactone (Singh *et al.*, 1992). Apart from this saussareal, a sesquterpenoid (Talwar *et al.*, 1992) has also been isolated.

Another genus *Arctium lappa*, of the same family commonly found in Himalayas and is closely related to *S. costus* in terms of chemical constituents and its traditional use. Both the plants contain guainolides but in the later, the guainolides are linked with a sulphur containing acetylenic compound viz. lappaphen-a and lappaphen-b (Washino *et al*, 1987). Apart from these *A. lappa* has also been reported to contain caffeic acid derivatives viz. chlorogenic acid, which is known to be an antioxidant compound (Shafiee *et al.*, 2002) and reported to possess anti-inflammatory and free radical scavenging effects, (Lin *et al*, 1996 & Lin *et al.*, 2000). Since both the plants are used as tonic, hence, we assumed that this activity may be due to the presence of chlorogenic acid (**1**). Thus an attempt was made to detect the presence of chlorogenic acid in *S. costus* and *A. lappa* by HPLC method. Apart from these, a comparative HPLC profiles of both the plant species are also made, so that, these profiles can also be used as distinguishing marker to differentiate between the two species widely used in Indian traditional system of medicine. The reproducibility of the results were also confirmed by using symmetry column. *S. costus* has not been reported to contain chlorogenic acid and hence we also report the presence of chlorogenic acid in *S. costus* for the first time, which was confirmed by HPLC.



(**1**) Chlorogenic acid

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Materials and Methods

Chemicals and reagents – Chlorogenic acid was purchased from Sigma Chemicals Ltd. (USA). Acetonitrile, phosphoric acid and water used were of HPLC grade. All the solvents used were filtered through 0.45 μm filter before use.

Plant material, extraction and sample preparation – Roots of *S. costus* were collected from Barsu, Uttarkashi district of Uttranchal, and *A. lappa* were collected from Baklood, Mandi district of Himachal Pradesh. The plants were authenticated by Dr. Vivek Kumar and the specimen voucher (voucher specimen number *S. costus* LWG91285 and *A. lappa* LWG91282) was deposited at the NBRI herbarium for future reference. Air dried (35-50°C) roots (25 g) were finely powdered and extracted with (3×50 ml) of methanol for 24 hrs. The extracts were concentrated under reduced pressure and dried in lyophilizer (Labconco, USA). The dried extract (10 mg) was taken and dissolved in 1 ml of methanol. Standard chlorogenic acid (1.0 mg) was dissolved in methanol (10 ml), which was used as stock solution. From this 5 concentrations (2-20 μg/ml) were prepared and extracts were filtered through a sample filtration kit (PTFE; Waters, Milford, USA).

Instrumentation – A waters modular HPLC system consisting of an automated gradient controller with two model 515 pumps, Rheodyne loop injector with 25 μl, a model 2996 Photo diode array detector, spherisorb C18 ODS2 column (5 μm, 4.6×250 mm, Waters) and Millenium 32 Chromatography Manager were used for analysis. The injector, gradient controller and chromatography manager were integrated to give reproducible results.

HPLC analysis – Solvent system consists of acetonitrile (A) and phosphoric acid (B) using a gradient elution in 0-13 min. of 10-22% of A, 13-14 min with 22-40% of A, a isocratic till 14.5 min., 14.5-15 min 40-10% of A and a isocratic with 10 % of A till 20 min. Flow rate of 1.5 ml/min was maintained and 20 μL of the sample was injected. Detection was made in 327 nm. The calibration curve for standard was plotted and was found linear in the range of 5-15 μg/ml.

Results and Discussions

The percentage of chlorogenic acid in methanolic extract was found to be 0.087%, 0.140% in *S. costus* and *A. lappa*, respectively. The retention time of the chlorogenic acid standard of 6.8 min. matches exactly with that of the chlorogenic acid in the extracts of *S. costus* R.T. 6.8 min. (Fig. 1) and *A. lappa*, R.T. 6.8 min. (Fig. 2). *A. lappa* also

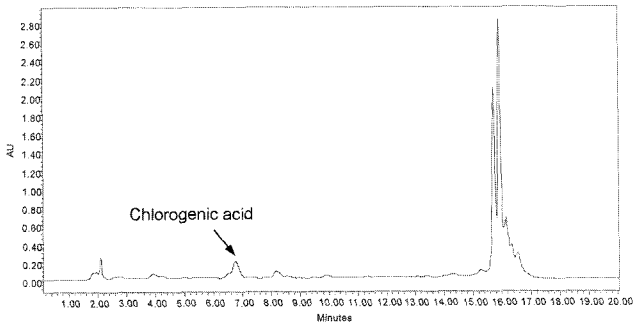

Fig. 1. HPLC chromotogram of a crude extract of *S. costus* anlysed using a symmetry C18 column clubbed with a binary gradient using acetonitrile: 0.1% phosphoric acid and detected using a PDA at 327 nm. Peak at 6.8 min was identified as chlorogenic acid.

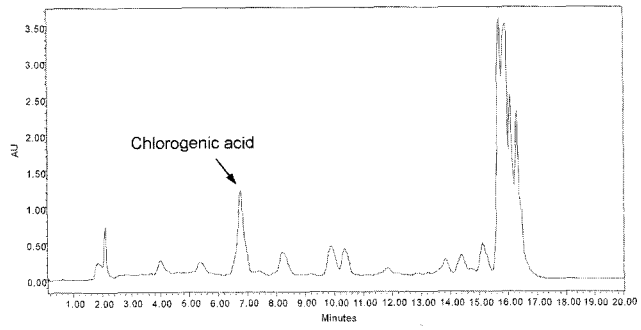

Fig. 2. HPLC chromotogram of a crude extract of *A. lappa* anlysed using a symmetry C18 column clubbed with a binary gradient using acetonitrile: 0.1% phosphoric acid and detected using a PDA at 327 nm. Peak at 6.8 min was identified as chlorogenic acid.

shows a number of the constituents that differ from *S. costus*.The profiles of the two extracts in the mentioned system shows clear difference between the two plants. *A.lappa* shows around 20 peaks while *S.costus* shows only 12 peaks with common peaks at RT, 6.8 (Chlorogenic acid), 2.1, 3.9, 9.9.15.9 and 16.3. The higher percentage of chlorogenic acid in *A. lappa* explains in part, its free radical scavenging potential. Since *S. costus* also contains substantial amount of chlorogenic acid, it may also possess free radical scavenging potential which is to be screened.

Bergeron *et al.*, (2000) has reported the HPLC method of phenolic compounds including chlorogenic acid from roots of *Echinacea angustifolia* and *Echinacea purpurea* using a gradient elution with acetonitrile and water. We, inorder to get a differentiating profile between the *S. costus* and *A.lappa*, modified the above mentioned procedure to include 0.1% phosphoric acid along with acetonitrile which enabled us to estimation of chlorogenic acid along with differentiating the two extracts. We also report the chlorogenic acid for the first time in *S. costus*.

S. costus and *A. lappa* has been used as a tonic in the Indian system of medicine and it has also been reported as

an anti-ulcer agent. As the role of free radicals in these diseases are well documented (Govindarajan *et al.*, 2003 and Halliwell *et al.*, 1997) the usage of these plants may be in part due to the free radical scavenging effect. Chlorogenic acid, being an antioxidant compound may be one of the factors responsible for the therapeutic efficiency of these plants. Thus chlorogenic acid can be used as a marker component in quality evaluation/standardization and also in differentiating the roots of *S. costus* and *A. lappa*. Also, HPLC profile assertains the presence of chlorogenic acid in *S. costus*. The HPLC profile of the two plants can also be used as a differentiation marker to identify the two plant species.

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