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Evaluation of Pharmacognostical Characters and Comparative Morphoanatomical Study of *Saussurea costus* (Falc.) Lipchitz and *Arctium lappa* L. Roots

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Abstract – *Saussurea costus* (Falc.) Lipchitz syn *S. lappa* C. B. Clarke (commonly known as 'Kuth') belonging to the family Asteraceae is a well known medicinal plant which finds wide usage in different indigenous systems of medicine of India, China, Korea & Tibet. In different folk medicines the roots of *S. costus* are used to treat various disorders like ulcer, stomachache, malaria, leprosy, dysentery and toothache. However due to over exploitation, it has become endangered and has become the concern of different governmental bodies in India. The increasing demand of this endangered Himalayan species has resulted in a situation where it is often substituted, knowingly or unknowingly, by other morphologically similar species. *Arctium lappa*, belonging to the same family, is one such plant that has often been found to be present in the market samples of 'Kuth'. The present study was thus carried out and morphoanatomical characters, physicochemical as well as chemical parameters were developed for proper identification of roots of *S. costus* and its differenciation from *A. lappa* as well as authentication of the commercial market samples. The detailed morphoanatomical studies revealed that roots of *S. costus* can be distinguished from *A. lappa* on the basis of some important microscopial characters eg. the schizogenous resin ducts observed in roots of *S. costus,* were absent in *A. lappa.*. Besides, the HPTLC fingerprint profile showed a distinct band at R_f. 0.72 in *S. costus,* which was totally absent in *A. lappa* and a band at R_f 0.64 in *A. lappa* which was absent in *S. costus* Chlorogenic acid, used as a chemical marker for HPTLC analysis, was estimated to be 0.077% in *S. costus* as compared to 0.107% in *A. lappa*. Thus these detailed pharmacognostical parameters can be successfully used to distinguish between roots of *S. costus* and *A. lappa*.
Keywords – *Saussurea costus*, *Arctium lappa*, 'Kuth', 'Jangali Kuth', Pharmacognosy, Chlorogenic acid

Introduction

Saussurea costus (Falc.) Lipchitz syn *S. lappa* C. B. Clarke. belonging to the family Asteraceae is a well known medicinal plant which finds wide usage in different indigenous systems of medicine of India, China, Korea & Tibet. Is is used mainly as antispasmodic in asthma, cough and cholera and also in chronic skin diseases and rheumatism (Chopra, *et al.,* 1956). In different folk medicines the roots of *S. costus* are used to treat various disorders like, ulcer, stomachache, malaria, leprosy, dysentery and toothache, (Kaul, 1941; Shah, 1982; Dhar, *et al.,* 1984; Rawat and Pangtey, 1987; Kala, *et al.,* 1999; Singh, 1999; Kapoor, 2001). The decoction of root is used to cure stomachache, toothache and typhoid fever (Nautiyal, *et al.,* 2003). This important medicinal plant grows in the sub- alpine regions of Jammu & Kashmir, HP & Uttarakhand at an altitude of 3200 - 3800 m. A review on this plant covering its botanical characters, chemistry & pharmacology along with its traditional and folk medicinal uses has recently been published by the authors (Pandey, *et al.,* 2007), which shows that world wide there has been a tremendous increase in interest in this plant. However due to over exploitation, it has become endangered and has become the concern of different governmental bodies in India. This critically endangered medicinal species is enlisted in Appendix I of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and is one of the 37 Himalayan endangered medicinal plants that have been prioritized for *in situ* and *ex situ* conservation (Kuniyal, *et al.,* 2005). Due to a resource base bottleneck, the Ministry of Commerce, Government of India, has prohibited export of 29 medicinal and aromatic species, including *S. costus*, either in crude form or in processed

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products (Anonymous 2000). The Planning Commission and the National Medicinal Plants Board (NMPB) of the Government of India have prepared a policy document on the promotional and commercial aspects of the medicinal plants sector. According to the Planning Commission, Government of India, quantity of *S. costus* required is to the tune of 0.43 tonnes per annum. The NMPB has prioritized 32 and Planning Commission has enlisted 24 medicinal plant species for research and development in order to meet the desired aim of the medicinal plant sector and both these lists include *S. costus* (Kala, *et al.,* 2006). The increasing demand of this endangered Himalayan species has resulted in a situation where it is often substituted, knowingly or unknowingly, by other species that are morphologically similar to it. One such plant is *Arctium lappa,* belonging to the same family. It also grows at similar altitudes. The roots of both have more or less similar morphological characters, which often create confusion in the correct identification of the two drugs, *S. costus* (commonly known as 'Kuth') and *A. lappa* (commonly known as 'Jangali Kuth'). The roots of *A. lappa* are diuretic and diaphoretic, used in gout and skin affections. Tincture of seeds is used for psoriasis and acne (Anonymous, 1992). Its roots are used in different preparations to treat ailments like gastric trouble and rheumatism (Rawat and Pangtey, 1987; Beigh, *et al.,* 2003). It is also used to repeal rodents (Koelz, 1979).

Arctium lappa, is closely related to *S. costus* in terms of chemical constituents. Roots of both the plants are rich in sesquiterpene lactones. However in *A. lappa* guainolides are linked with a sulphur containing acetylenic compound. Both have also been reported to contain chlorogenic acid (Pandey, *et al.,* 2004), which is a known antioxidant (Shafee, *et al.,* 2002) and is reported to possess anti-inflammatory and free radical scavenging activities (Lin, *et al*, 1996; Lin, *et al.,* 2000; Chen, *et al.,* 2004).

The close similarities between *S. costus* and *A. lappa* have resulted in a dire need to effectively distinguish

Table 1. Comparative botanical characters of root of *S. costus* and *A. lappa*

	S. costus	***A. lappa***
Macroscopic characters	• Roots stout, dark brown or grey in colour with longitudinal ridges and rough reticulate surface. • Dry roots longitudinally wrinkled and dark yellowish brown in colour. • Fracture short, texture rough • Taste bitter and pungent odour characteristic and pleasant. • Transversely cut root shows the outer cork gray in colour and inner surface light brown in colour.	• Roots fusiform, externally brown or light yellowish brown in colour. • Dry roots longitudinally wrinkled crown annulate and frequently showing wooly tuft of leaf remnants. Fracture short and horny texture rough. Taste mucilaginous, no characteristic odour. • Transversely cut root shows dark cambium separates the thick bark.
Microscopic characters		
Phellem (Outer cork)	• 3 - 5 layered, suberised radially arranged somewhat tangentially elongated brown colourd, about 20 - 60 µm length and 15 - 30 µm width.	• 3 - 5 layered, suberised irragular shaped cork cells, about 22 - 75 µm length and 15 - 30 µm width.
Phellogen (Cork cambium)	• 1 - 2 layered, thin walled, rectangular and tangentially elongated. Cells about 15 - 48 µm long and 9 - 30 µm in width.	• 1 - 2 layered cells about 28 - 66 µm long and 9 - 30 µm in width.
Phelloderm (cortex)	• 5 - 9 layered parenchymatous followed by 15 - 25 layers of parenchymatous cortex, cells 18-85 µm long and 15-65 mm in width.	• 8 - 10 layered followed by 25 - 40 layers of parenchymatous cortex. Cells polygonal with intercellular space about 20 - 76 µm length and 15 - 50 µm in width.
Secondary phloem	• About 1350 - 1530 µm broad and up to 100 layers of phloem tissue.	• 600 - 650 µm broad.
Medullary rays	• Multiseriate 16 - 40 µm in diameter.	• 15 - 40 µm in diameter.
Vessels	• With broad lumen, solitary or in a group of 2 - 6 layered	• With broad lumen solitary or in a group of 2 - 8 layered
Fibres	• Lignified 240 - 415 µm long	• Non lignified, 288 - 729 µm long
Schizogenous resin ducts	• Large number of cavities present.	• Absent
Powder characteristics	• Dark brown or yellowish brown. • Characteristic odour. • Bitter and pungent in taste. • Xylem vessels elongated or drum shaped with spiral scalariform and reticulate secondary wall thickening. • Orange or brownish resin canals present.	• Yellowish brown in colour. • Not so characteristic odour. • Slightly bitter, and mucilaginous in taste. • Xylem vessels with spiral, sclariform and reticulate lignified secondary wall thickening. • Resin cells/canals are absent.

between the two. However, no detailed pharmacognostical studies of these two important Himalayan drugs are on records excepting a Germen monograph on *S. costus* only (Bruchhausen, *et al.,* 1994). Therefore, taking in to account the above considerations, the present study was carried out so as to define morphoanatomical characters, physicochemical as well as chemical parameters for proper identification of roots of *S. costus* and *A. lappa* as well as authentication of the commercial market samples. Chlorogenic acid found present in both the plants was used as chemical marker for HPTLC analysis.

Experimental

The roots of *S. costus* were collected from Uttarkashi District of Uttarakhand during September 2000 and *A. lappa* from Baklood (Mandi district) of Himachal Pradesh during April 2000. The herbarium specimens were prepared and identified as per standard herbarium procedure (Jain & Rao, 1977) and finally deposited in the Herbarium of National Botanical Research Institute, Lucknow [voucher specimen number LWG 91285 (*S. costus*) and LWG 91282 (*A. lappa*)] for future reference. Sectioning was done in transverse and longitudinal planes on rotary Microtome. Different commercial samples of 'Kuth' were collected from five different raw herbal drug markets viz. Bangalore, Chennai, DehraDun,, Joginder Nagar and Palampur of India. Phytochemical studies were performed with the shade dried powdered material. Precoated HPTLC silica gel G 60 F_{254} plate (Merck) and Chlorogenic acid purchased from Sigma chemical Ltd. (USA) were used.

Physicochemical evaluations – Air-dried materials were used for quantitative determination of ash values, sugars, tannins, alcohol and water-soluble extractives and successive extractive percentages. The recommended procedures were followed for calculating ash values *viz.,* total ash and acid insoluble ash percentages (Anonymous, 1996), tannins (Anonymous, 1984) and total sugars (Montgomery, 1957).

HPTLC studies

Preparation of samples – Air dried roots (5 g) of both as well as commercial samples were coarsely powdered

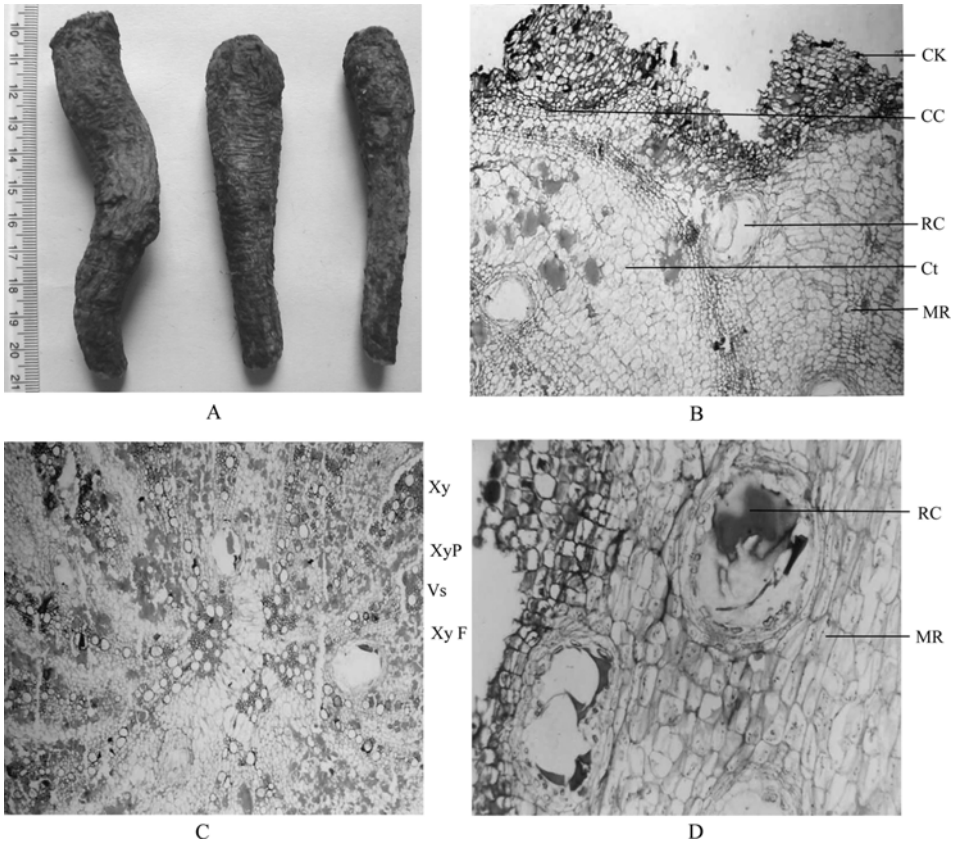

Fig. 1. Macro and microscopy of *S. costus* root. A: root samples; B: T.S. showing cortical region (× 40); C: T. S. showing central region (× 40); D: L.S. showing cortical region and resin canal (× 100).

Abbreviations: T. S.: transverse section, L.S.: longitudinal section, CK: Cork cells, CC: Cork cambium, Ct: Cortex, MR: Medullary rays, RC: Resin canal, Vs: Vesssels, Xy**:** Xylem, XyF: Xylem fibre, XyP: Xylem parenchyma.

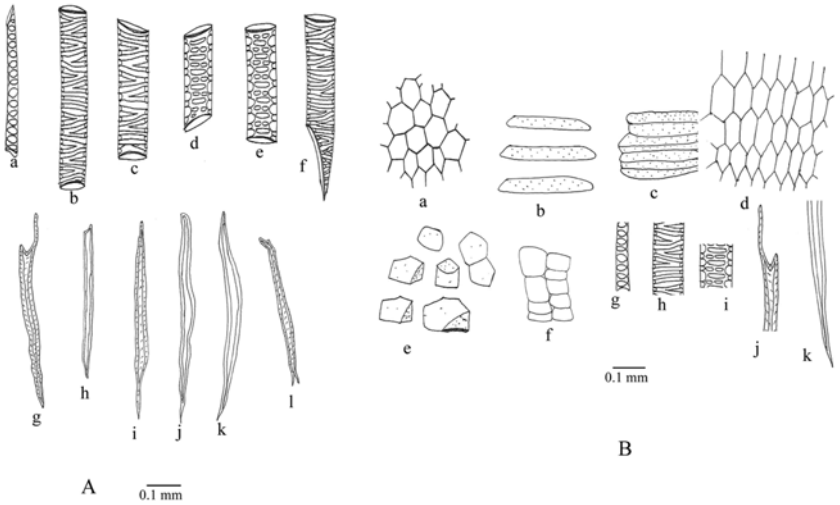

Fig. 2. A: Macerated elements; B: Powder charactersistics of the *S. costus* root.
Abbreviations: Macerated elements- a- f: Vessels, g, h, i, & l: tracheids and tracheidal fibres, j -k : fibres. Powder- a: cork cells, b-d: parenchyma cells, e: inulin crystals, f: cork cells, g - i: vessels, j: tracheidal fibre, k: fiber.

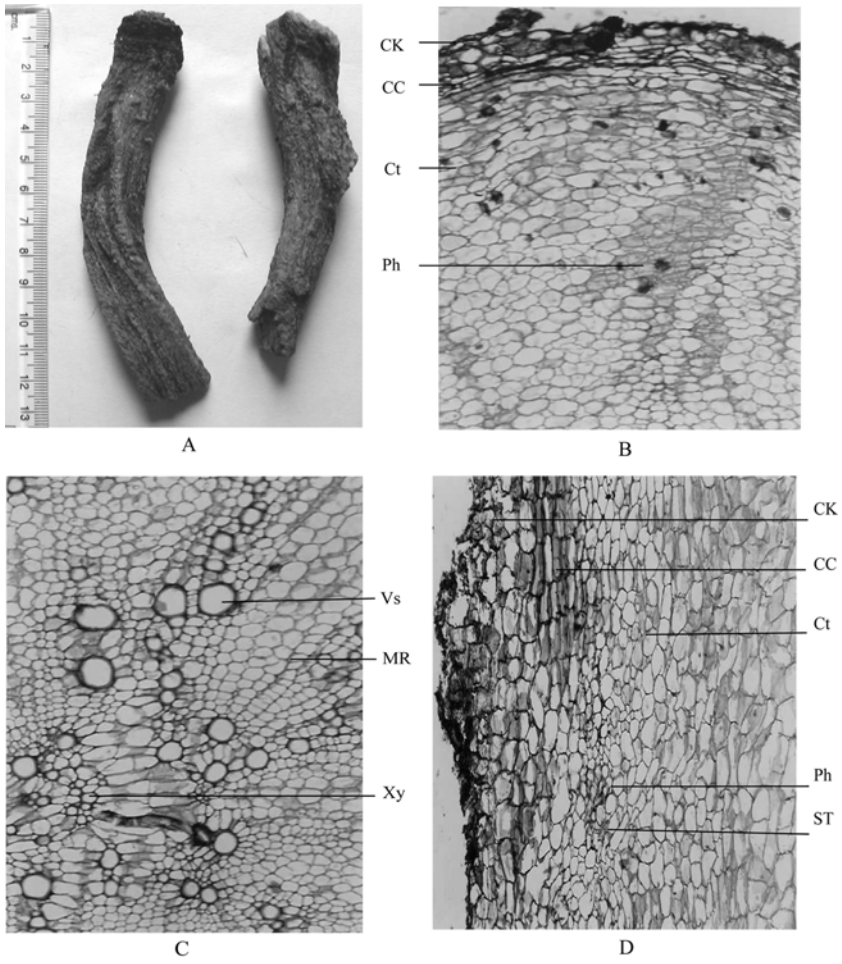

Fig. 3. A: Macro and microscopy of *A. lappa* root. A: root samples; B: T. S. showing cortical region (× 100); C: T. S. showing central region (× 100); D: L. S. showing cortical region (× 100).
Abbreviations: CK: Cork cells, CC: Cork cambium Ct: Cortex, Ph: phloem, Ca: cambium, ST: Sieve tube, Vs: Vesssels, MR: Medullary rays, Xy: Xylem.

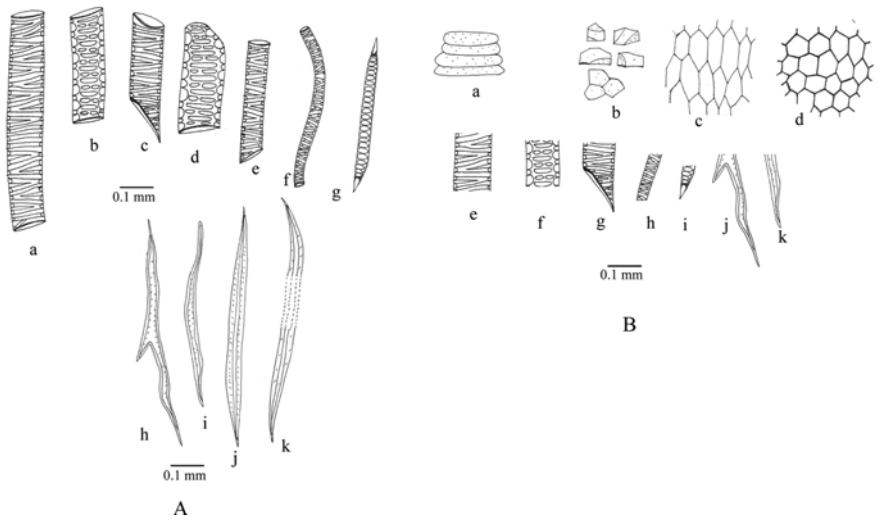

Fig. 4. A: Macerated elements; B: Powder charactersistics of the *A. lappa* root.
Abbreviations: Macerated elements- a-g: vessels, h-i: tracheidal fibre, j-k: fibres. Powder- a: parenchymatous patches, b: inulin crystals, c: parenchymatous cells, d : cork cells, e - i: vessels, j - k: tracheidal fibres.

and extracted with methanol (3 × 100 mL) for 24 hrs. The extracts were concentrated under reduced pressure and dried in lyophilizer (Labcono, USA). The dried 10 mg extracts of each were dissolved in 1 ml of methanol and 15 µL of each was applicated with a Linomat IV applicator on to a HPTLC plate (merck, 0.2 mm thickness, F_{254}). Chlorogenic acid was used as a chemical marker. After drying, the plate was developed with Ethyl Acetate: Formic Acid: Acetic Acid: Water (10 : 1 : 1 : 2.6) up to 75% of the plate.

Detection – The developed plate was observed under UV λ 254 and UV 366 nm. It was further scanned at λ 330 nm (λ max for chlorogenic acid). After scanning, the plate was derivatized using Ferric chloride reagent to detect the compounds.

Results and Discussion

Morphoanatomical characters of the roots – The morphological and anatomical characters of both *S. costus* and *A. lappa* roots were studied and Table 1 (Fig. 1 - 4) gives their comparative view.

Physicochemical study – The physicochemical values like total ash, acid insoluble ash, alcohol and water-soluble extractive were calculated. Besides, the total percentage of starch, tannin, sugars and successive soxhlet extractives using hexane, chloroform, acetone, alcohol and water were also calculated and the results documented (Fig. 5& 6).

HPTLC studies- Qualitative as well as quantitative analysis – HPTLC of the extracts of both roots (*S. costus* and *A. lappa*) and commercial samples were performed in the solvent system Ethyl Acetate: Formic Acid : Acetic

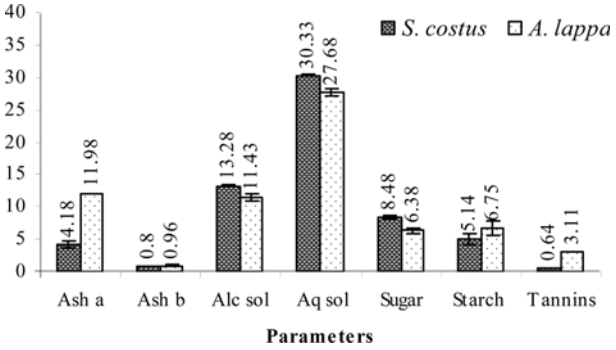

Fig. 5. Comparative estimation of physicochemical parameters of *S. costus* and *A. lappa* roots (in %).
Ash a: total ash; Ash b: Acid insoluble ash; Alc sol: Alcohol soluble extractive; Aq sol: Water soluble extractive.

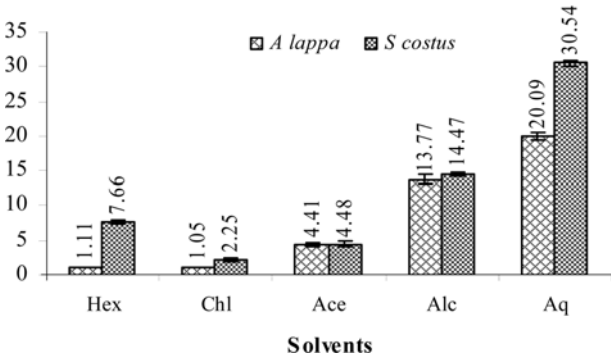

Fig. 6. Successive Soxhlet extractive values (in %) of *S. costus* and *A. lappa* roots.
Hex: Hexane; Chl: Chloroform; Ace: Acetone; Alc: Alcohol; Aq: Water.

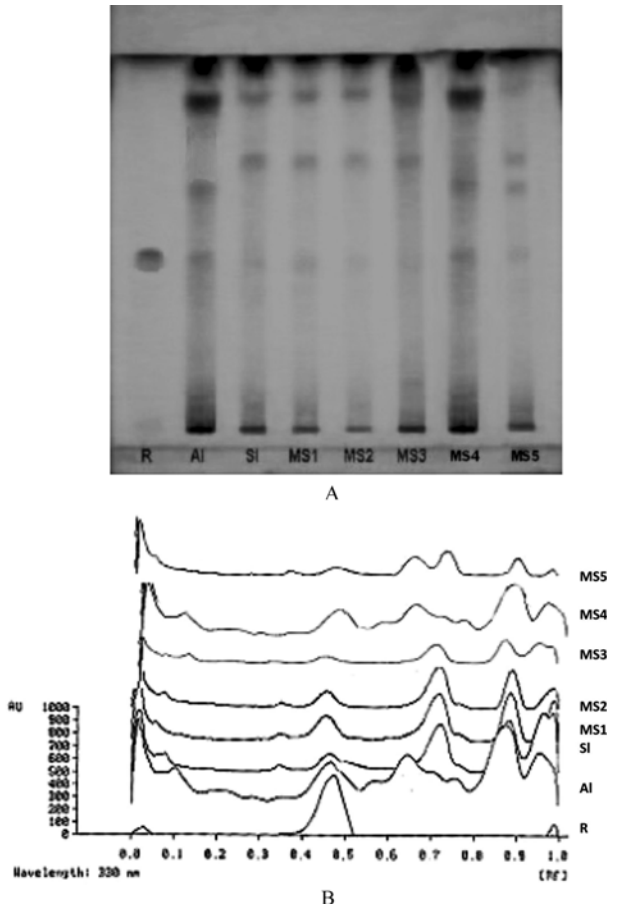

Fig. 7. A: Comparative HPTLC finger print profile of *S. costus, A. lappa* and commercial samples (Under UV 254 nm); B: densitometric scan at λ 330 nm.

R: Chlorogenic acid; Al: *Arctium lappa;* Sl: *S. costus;* MS1: Bangalore market; MS2: Chennai market; MS3:DehraDun market; MS4: Joginder Nagar market; MS5: Palampur market.

Acid : Water (10 : 1 : 1:2.6), Chlorogenic acid was used as a standerd and the percentage of the same was calcuted in *S. costus*, *A. lappa* and commercial samples.

The qualitative densitometric HPTLC fingerprint profiles showed a number of similar bands at R_fs 0.08, 0.34, 0.46 and 0.88 between the two species, indicating similarity in the chemical constituents. However, there was a distinct band at R_f. 0.72 in *S. costus,* which was totally absent in *A. lappa* and a band at R_f 0.64 was observed in *A. lappa* which was absent in *S. costus*. Incidentally, these were found to be the major bands, and thus distinguishing markers between the two species. Thus HPTLC profile as such can be used as a parameter for standardization and quality evaluation of *S. costus* It can also be used to differentiate the two species *S. costus* and *A. lappa* as evident from Fig. 7. Chlorogenic acid was estimated and was used as a chemical marker to differentiate between the two species *S. costus* and *A. lappa.* The percentage of chlorogenic acid was found to be 0.077% in *S. costus* which was quite less as compared to 0.107% in *A. lappa*. Though *S. costus* is considered as an official drug the high percentage of chlorogenic acid indicates that it may not be the only active principle responsible for the therapeutic efficacy of the traditional uses of the plant. The therapeutic efficacy of the *S. costus* may be due to the other secondary metabolites present. The different commercial samples of 'Kuth' were authenticated using above pharmacognostical parameters including HPTLC fingerprint profile of 'Kuth' and 'Jangali kuth' and the commercial samples were found to consists only of *S. costus* in Dehra Dun, Bangalore and Chennai market, *A. lappa* in Joginder Nagar herbal drug market and mixture of *A. lappa* and *S. costus* roots in Palampur market.

Conclusions

The detailed morphoanatomical study revealed that *S. costus* can be distinguished from *A. lappa* on the basis of some important microscopial characters. The roots of *S. costus* and *A. lappa* showed certain minor variations in their morphological characters. For example, outer surface of roots of both the species are rough. However, the colour of the outer bark of *S. costus* is dark yellowish brown or greyish, while it is brown or light yellowish brown in *A. lappa.* Similarly, the transverse cut surface of *S. costus* shows that the outer cork is grey and inner cork is light brown in colour whereas, in *A. lappa* the dark cambium separates the thick bark. On the contrary the anatomical studies of both the species showed certain variations *viz*., the size of cork cells, the phloem region of *S. costus* is broad, the fibres are thick walled and lignified in *S. costus* as compared to *A. lappa,* where these are thin walled, non-lignified and longer. The size of vessels are longer in *A. lappa* as compared to *S. costus*. The schizogenous resin ducts were also observed in roots of *S. costus,* which were absent in *A. lappa.* Besides, the xylem fibres are much more in number in the roots of *S. costus* as compared to *A. lappa.*

Since no methods which are based on chemical constituents are available, development of chromatographic fingerprints and assay methods of the bioactive constituents can contribute significantly to the quality evaluation of *S. costus* and *A. lappa* . Thus, the HPTLC fingerprint profile showing a distinct band at R_f. 0.72 in *S. costus,* which was totally absent in *A. lappa* and the band at R_f 0.64 observed in *A. lappa* which was absent in *S. costus* could be used as distinguishing characters between the two

species. Besides, studies on quantitative estimation of chlorogenic acid in *S. costus* and *A. lappa* by HPTLC showed it to be 0.077% in *S. costus* as compared to 0.107% in *A. lappa*. These results were similar to those obtained by HPLC earlier (Pandey, *et al.,* 2004). Thus HPTLC method can be used as a faster and economical method for quality evaluation.

Study of the commercial samples confirms that *S. costus* roots are being knowingly or unknowingly substituted with *A. lappa* which is more or less morphologically similar. These finding thus necessitate the development of pharmacognostical parameters for differentiating between these two species.

It can thus finally be concluded that the above detailed pharmacognostical parameters can be successfully used to distinguish between roots of *S. costus* and *A. lappa.*

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