Comparative pharmacognostical studies of two medicinally important Indian 
Evolvulus species

Saba Irshad1,2, Siddhartha Pragyadeep1, Ajay Kumar Singh Rawat1, PK Misra2 & Sayyada Khatoon1* 
1Pharmacognosy and Ethnopharmacology Division, CSIR-National Botanical Research Institute, Post Box No. 436, Rana Pratap Marg, Lucknow -226001, India; 2Department of Botany, University of Lucknow, Lucknow- 226007, India 
E-mails: sayyadak@yahoo.com; sayyadak@gmail.com; sayyadak@nbri.res.in

Received 18 November 2014, revised 19 December 2014

The genus Evolvulus consists of small herbs or under shrubs distributed in the tropical and warm temperate regions of the world. Two species of this genus have been reported from India, viz. E. alsinoides L. and E. nummularius L. (Convolvulaceae). Both the species are traditionally being used as nerve tonic and for the treatment of skin diseases. The current study was carried out to provide comparative macro-microscopy, physicochemical parameters and TLC fingerprint profiles of aforesaid Indian Evolvulus species. The microscopy showed anisocytic stomata in E. alsinoides while diacytic and paracytic stomata in E. nummularius; presence of stellate trichomes, characteristic pith with spindle shaped deposition of calcium oxalate crystals and starch grains only in E. nummularius. Comparative TLC profile showed presence of some common as well as differentiating bands in methanolic extract of Evolvulus sp. However, the chemical markers, viz. ferulic acid, caffeic acid, β sitosterol and lupeol were present in both the species. The macro-microscopy and TLC profiles may play an important role for identification and quality evaluation of two medicinally important Evolvulus species.

Keywords: Evolvulus alsinoides, Evolvulus nummularius, Macro-microscopy, Pharmacognosy, TLC

IPC Int. Cl.3: A61K, A61K 36/00, A61J 3/00

Convolvulaceae, commonly known as morning glory family, represented by 60 genera and more than 1,650 species and many of them have medicinal properties. Evolvulus, is a genus of this family, consists of small herb or under shrubs distributed in the tropical and warm temperate regions of the world. Although more than 70 species are reported from all over the world but only two species of this genus have been reported from India, viz. E. alsinoides L. (EA) and E. nummularius L. (EN)1. Both the species are reported for their therapeutic claims in the traditional systems of medicines and in folklores. EA is known by different vernacular names, viz. Shankhpushpi, Shnkha valli, Vishnugandhi, Vishnukarandi, Vishnuka ranta, etc. Traditionally it is used all over India for the treatment of various ailments, viz. fever, cough, cold and jaundice, as nerve tonic, hysteria, to cure burns, cuts, wounds and scorpion stings, etc.1-2. Pharmacologically it is reported to possess antulcer and anticatatonic3; immunomodulator, antiinflammatory4 and nootropic5 activities. Another species EN also called by various names, viz. Aakhukarni, Muusaakarni and Chhinipatra6. Traditionally the whole plant is used as a medicine for hysteria, to cure burns, cuts, wounds and scorpion stings7. Pharmacologically it is reported for wound healing8, as anthelmintic9, poor sedative and anticonvulsant10. On account of aforesaid medicinal properties, quality control markers of EA and EN are required. Preliminary pharmacognostical data reported for EA11 only.

EA contains several alkanes, alkaloids, fatty acids, flavonols, flavonoids, organic acids, phenolics, phytosterol, saponins, and tannins12. Some of them are betain, shankhpushpine and evolvine13; scoptoletin, scopolin, umbelliferon, ferulic acid easters, kampferol-7-O-β-glucopyrenoside and quecitin-3-O-β glucopyrenoside and caffeic acid14. EN reported to contains β-sitosterol and its glucoside, stigmasterol, ursolic acid, oleanolic acid, palmitic acid and nummularic acid15.

Therefore, present study is plan out to investigate comparative macro-microscopical characters, physicochemical parameters and TLC fingerprinting for EA and EN with the aim to set the quality control parameters of these two important Indian medicinal plants.

*SCorresponding author
Materials and methods

Collection and processing of plant material

The selected plant species of *Evolvulus* were collected from Chitrakoot and Lucknow in July and August 2013, their herbarium specimens were prepared as per standard herbarium procedure and deposited in the herbarium of CSIR-National Botanical Research Institute, Lucknow wide voucher specimen number LWG-30 and LWG-33 for *E. alsinoides* and *E. nummularius*, respectively.

Macro-microscopical studies

The macroscopy of *Evolvulus* species was described with the help of Floras. Qualitative and quantitative microscopy, powder studies and florescent analysis were done according to the standard methods.

Physicochemical studies

Total ash, acid insoluble ash, alcohol and water soluble extractives were calculated as per Pharmacopoeial methods. Sugar and starch were also estimated according to Montgomery method.

Preparation of sample solutions for TLC finger printing

For TLC studies, plants were air-dried at room temperature in the shade and were used for solvent extraction. The whole plant of EA and EN ground to coarse powder and placed in appropriately sized volumetric flasks. 25 mL methanol was added to 4 gm of powder of each plant, shaken on shaker for 2 hrs, kept at rest overnight. The methanolic extracts were filtered through Whatmann No. 1 filter paper. The procedure was repeated thrice with methanol (25 mL) at room temperature (25°C ± 2°C). The extracts were concentrated under reduced pressure at a temperature of 45±2°C. Accurately weighed 10 mg of the extract was dissolved in 1 mL methanol, and filtered through a 0.45 μm filter membrane, the filtrate was used as sample solution. 1 mg each of ferulic acid, lupeol and β-sitosterol were dissolved in 10 mL methanol to get 0.1 mg/ mL solution of standard markers.

TLC finger printing

TLC was performed on 10x 20 cm silica TLC aluminium sheet, coated with 0.2 mm layer of silica gel containing UV 254 fluorescent indicator (S D Fine Chemicals, India). Samples (20 μL) and standards (10 μL) were applied to the plates by means of a Camag (Switzerland) Linomat 5 sample applicator. The plates were developed to a distance of 8 cm from the lower edge of the plate with 20 mL toluene-ethyl acetate-formic acid (8.5:1.5:0.1 v/v/v) as mobile phase, in a Camag twin-trough chamber, previously saturated with mobile phase vapor for 30 min at 25±2 °C. After removal from the chamber, plates were completely dried in air at room temperature (25±2 °C) and documented under UV 254 nm and UV 366 nm. The plates were dipped in anisaldehyde sulphuric acid reagent, dried and heated at 110±2 °C for 5 min and documented in visible light after derivatization.

Results and discussion

Comparative macroscopy of two studied *Evolvulus* species (Fig. 1A & 2A) has been shown that root is straight; cylindrical; 8 to 10 cm long, rooting at the nodes in both the species. Stem-numerous; prostrate; often more than 30 cm long; usually clothed with long silky hair in EA although in EN stem are not covered with silky hairs. In EA leaf-simple, alternate, sessile, lamina oblong, lanceolate, epicatele usually acute at the base, shallowly, emarginate and mucronate at the apex while in EN, leaf- simple; alternate, petiolute, lamina circular or circular-obovate, obtuse, base truncate to subcordate, glabrous or sparingly pubescent beneath. In EA, flower-one to three with peduncle, axillary; calyx-densely silky, 5 lobed, acute, lanceolate; corolla- blue-purple, five, shallow lobed, funnel shaped with small white eye inside but in EN, flowers-solitary or rarely paired, pedunculate, axillary; calyx-ovate to elliptic ovate, acute; corolla-white, subrotate, deeply 5 lobed, lobes obovate. Capsule- globose, 4 valved, 3 mm in diameter, seeds brownish in EA and bivalve, 3–4 mm. in diameter, seeds brown to black in EN. The characteristic microscopic features are - anisocytic stomata in EA while diacytic to paracytic stomata in EN; simple and glandular trichomes in EA and stellate trichomes in EN; tenniniferous cells in EA while schizogenous mucilaginous canals and calcium oxalate crystals in EN. Further, pith is parenchymatous in the transverse view of both stem but embedded with spindle shaped deposition of starch grains and calcium oxalate crystals only in EN.
microscopical details of EA and EN are given in Table 1 and shown in Figs. 1 & 2.

Air dried material was used for quantitative determination of physicochemical values. Extractive values (alcohol and water soluble), ash values (total and acid insoluble ash), total sugar and starch content were repeated for six times and mean values ±SD was presented in Fig. 3. Higher water extractive value of EN indicating the possibility of considerable amount of polar compounds and also high starch content in EN showed the high rate of photosynthesis and low growth rate. Total ash value of EA was relatively low, which may be due to low content of carbonates, phosphates, silicates and silica. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards.

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents develops different colour in the visible light, however, ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine). If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation. The results of fluorescence study of whole plant powder using different reagents are given in Table 2.

TLC fingerprint profile showed similar and differentiating bands in Figs. 4A, B & C. Common bands at Rf 0.12, 0.23, 0.30, 0.59 (under UV 366 nm) at Rf 0.19, 0.30, 0.62, 0.75 (under UV 254 nm) and at Rf 0.30, 0.48, 0.62 and 0.75 (under visible light after derivatization) were observed in both the species. However, the identifying blue fluorescent band at Rf 0.88 under UV 366 nm and greyish blue band at Rf 0.71 after derivatization were observed only in EN. Similarly,
the characteristic blackish grey band at Rf 0.68 under UV 254 nm and after derivatization while another pulish grey band at Rf 0.79 after derivatization in EA were observed (Fig. 4, Table 3). Three chemical markers viz. ferulic acid, β-sitosterol and lupeol showed simultaneous bands at Rf 0.30, 0.48, 0.62, respectively (Fig. 4) in both the species.

Quality of herbal drug in term of active principal and their efficacy necessitates the need of quality control studies of raw drug materials. Global marketing has also created awareness towards validation of plant based drug to maintain the quality, safety and efficacy. As these two medicinally important *Evolvulus* species being a nerve tonic in traditional bsystems of medicine, have a potential to
Fig. 2—Microscopy of the whole plant of *E. nummularius*. A: whole plant, B: T.S. Root, C: T.S. Stem, D: T.S. leaf passing through lamina; E: T.S. Leaf passing through midrib; F: Powder - a: prismatic crystals, b-d: xylem vessels, e: stellate trichomes, f: simple trichome, g: epidermal cells with stomata. Abbreviations: ck, cork; ct, cortex; gt, glandular trichome; hyp, hypodermis; lepi, lower epidermis; sc, schizogenous mucilaginous canal; p, pith; pal, palisade layer; ph, phloem; sg, starch grains; sm, spongy mesophyll; uepi, upper epidermis; v, vessels; xy, xylem.

Fig. 3—Physicochemical parameters of *E. alsinoides* and *E. nummularius* as the mean value ± standard deviation (n≥ 6)
Table 2—Florescence analysis of the powder of whole plant of *E. alsinoides* and *E. nummularius*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagent</th>
<th>Under UV 366</th>
<th>Under UV 254</th>
<th>Visible light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EA</td>
<td>EN</td>
<td>EA</td>
</tr>
<tr>
<td>1</td>
<td>Powder+1M NaOH</td>
<td>DB</td>
<td>DG</td>
<td>DB</td>
</tr>
<tr>
<td>2</td>
<td>Powder+CH₃COOH</td>
<td>LB</td>
<td>DB</td>
<td>LB</td>
</tr>
<tr>
<td>3</td>
<td>Powder+5% I₂</td>
<td>LG</td>
<td>BG</td>
<td>BG</td>
</tr>
<tr>
<td>4</td>
<td>Powder+5% FeCl₃</td>
<td>DG</td>
<td>RB</td>
<td>DG</td>
</tr>
<tr>
<td>5</td>
<td>Powder+Conc. HNO₃</td>
<td>DB</td>
<td>LB</td>
<td>DB</td>
</tr>
<tr>
<td>6</td>
<td>Powder+Conc. NH₃</td>
<td>LG</td>
<td>DG</td>
<td>LG</td>
</tr>
</tbody>
</table>

Abbreviations: Dark Brown (DB), Dark Green (DG), Yellowish Green (YG), Light Brown (LB), Light Green (LG), Raddish Brown (RB), Brownish Green (BG).

Table 3—Comparative Rf details of *E. alsinoides* and *E. nummularius*

<table>
<thead>
<tr>
<th>Sample → Colour</th>
<th>Under UV 366</th>
<th>Under UV 254</th>
<th>After derivatization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EA</td>
<td>EN</td>
<td>EA</td>
</tr>
<tr>
<td>0.12 Blue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.19 Black</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.23 Blue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.30 Dark Blue/Black</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.48 Greyish blue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.59 green</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.62 purple</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.68 black</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.71 Greyish blue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75 Blackish green</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.79 Purplish grey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.88 Blue</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4—TLC profile of methanolic extract of two *Evolvulus* species. 1, *E. alsinoides*; 2, *E. nummularius*; R1; Lupeol; R2, β-sitosterol; R3, Ferulic acid. A: visualization under UV 254nm, B: visualization under UV 366nm, C: visualization under visible light after derivatization.

develop neuroprotective drug. These parameters can be utilized as reference for setting limits for the quality control of drugs/formulation where *E. alsinoides* and *E. nummularius* are being used or would be explored for new drugs by pharmaceutical industries.

**Conclusion**

From the present studies, it can be concluded that the characteristic macro-microscopical features, physicochemical parameters and distinguishing bands in the TLC profiles are very important as quality control markers of medicinally important Indian *Evolvulus* species.

**Acknowledgement**

The Authors are thankful to Director CSIR-National Botanical Research Institute, Lucknow for providing lab facility for this research work and first author is also thankful to Indian Council of Medical Research, New Delhi for financial support.

**References**

10 Chitralekha C, Dey PK & Dey CD, Pharmacological screening of Valeriana wallichii Lallemantia royleana, Breynia rhamnoides and Evolvulus nummularius for sedative and anti-convulsant principles, Naturwissenschaften, 51 (1964) 411.


