Natural Product Sciences
13(4) : 273-278 (2007)

Pharmacognostical Identification of Stem and Root of *Ipomoea quamoclit* (Linn.)

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Abstract – This paper presents a detailed pharmacognostical study of the stem and root of the crude drug *Ipomoea quamoclit* Linn. (Convolvulaceae). Morphoanatomy of the stem and root have been studied with the aim to aid pharmacognostic and taxonomic species identification using light and confocal microscopy, WHO recommended physico-chemical determinations and authentic phytochemical procedures. The physico-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of stem and root of *I. quamoclit* and may possibly help to differentiate the drug from its other species.
Keywords – *Ipomoea quamoclit,* Convolvulaceae, pharmacognosy, stem and root

Introduction

Ipomoea quamoclit Linn. (Convolvulaceae) is commonly known as Cypress Vine or Indian Pink and is often cultivated as an ornamental plant. A perusal of the reports reveal that the plant is used in Queensland as a purgative, for snakebite and as a snuff. The pounded leaves are applied as poultice to bleeding piles and at the same time the juice is mixed with hot ghee and given internally. The leaves are used as a plaster for carbuncles. In Spain, the powdered root is given as a sternutatory (The Wealth of India, 1998) and the pounded leaves are applied in haemorrhoids, ulcers and breast pain (Agarwal, 1997). In the Siddha system of medicine the leaves are used in the treatment of diabetes and piles (Yoganarasimhan, 2000) and the leaf and stem decoction is used in fever (Loustalot and Pagan, 1949). The leaves are used for treating carbuncles and in vomiting and are considered as cooling. In Ayurveda the leaves are used for stabilizing the gravid uterus (Kirtikar and Basu, 1991).

In spite of the numerous medicinal uses attributed to this plant, there are no pharmacognostical reports on the stem and root of this plant. Hence, the present investigation is an attempt in this direction and includes morphological and anatomical evaluation, determination of physico-chemical constants and the preliminary phytochemical screening of the different extracts of *I. quamoclit*.

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Experimental

Plant material – The stems and roots of *I. quamoclit* were collected from Erode, Tamil Nadu, India during the month of September, 2005. The botanical identity of the plant was confirmed by Dr. P. Jayaraman, Botanist, Medicinal Plant Research Unit, Chennai, Tamil Nadu. A voucher specimen (PP 545) has been deposited at the Museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal.

Chemicals and instruments – Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Solvents *viz.* ethanol (95%), hexane, petroleum ether, diethyl ether, chloroform, acetone, n-butanol and reagents *viz.* phloroglucinol, glycerin, HCl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Macroscopic and microscopic analysis – The macroscopy and microscopy of the stem and root were studied according to the method of Brain and Turner (1975a). For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (1940). The micropowder analysis was done according to the method of Brain and Turner (1975b) and Kokate (1986a).

Physico-chemical analysis – Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed (Indian Pharmacopoeia, 1966) and the WHO guidelines

on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992). Fluorescence analysis was carried out by the method of Chase and Pratt, 1949 and Kokoski *et al*., 1958.

Preliminary phytochemical screening – Preliminary phytochemical screening was carried out by using standard procedures described by Kokate, 1986b and Harborne, 1998.

HPTLC studies – Qualitative densitometric HPTLC analysis was performed for the development of characteristic finger print profile for successive petroleum ether (PE) and chloroform ($CHCl_3$) fractions of stems. 10 µL of the sample solutions were applied and the plates were developed in toluene-solvent ether (8.5 : 1.5) and $CHCl_3$-MeOH (8 : 2) respectively for PE and $CHCl_3$ fractions. Developed plates were then scanned densitometrically at various wavelengths. R_f values, peak area and spectrum of each peak were determined for these fractions. The presence of amyrin acetate in EtOH extracts and petroleum ether fraction were confirmed by co-chromatography with authentic sample (Sigma Chemicals Company, USA). Quantification of amyrin acetate was carried out for the PE fraction of the stem. The linearity of the HPTLC method was investigated for amyrin acetate in the range of 100-1000 ug/mL at five concentration levels using the Camag Linomat V applicator onto the precoated silica gel plate (Merck). The plate was then eluted with toluene-solvent ether (9 : 1). After elution the plate was sprayed with 10% methanolic H_2SO_4, heated at 105 °C for 5 mins and scanned densitometrically using Camag TLC scanner 3 at 600 nm. The percentage of amyrin acetate in the petroleum fraction was calculated by calibration using peak height and peak area ratio.

Results and Discussion

Macroscopic characters – The stem is a slender, twining and glabrous vine; size- 4 m or more, light green in colour with agreeable odour and slight bitter taste, fractured surface showing fibrous nature. Root is cylindrical and slightly tapering, branched and shows fibrous fracture, 2 - 5 cm long and 2 - 4 mm thickness. The inner wood is yellowish brown in colour.

Microscopic characters

Transverse section of stem – The stem is circular in cross-sectional view with broad, thick ridges and shallow wide furrows. The vascular cylinder is thin and the pith is wide. The stem is 2.5 mm in diameter. The stem consists of a thick epidermis measuring about 20 µm in radial plane. The cortex is narrow, homogeneous and parenchymatous. Along the ridges, the cells are collenchymatous. The circular, fairly wide secretory canals are distributed in the cortex along the circumference and are about 50 µm in diameter. It is surrounded by a layer of epithelial cells, which are not distinct from the neighboring cells. The vascular cylinder is closed, uniformly thin and in the initial stage of secondary growth. It consists of a thin continuous zone of phloem. On the outer surface of the phloem cylinder is a thin discontinuous layer of sclerenchymatous cells. The xylem consists of several radial files of xylem elements. Along the inner circumference of the xylem cylinder and on the periphery of the pith, small groups of inner phloem or medullary phloem are seen (Figs. 1A and 1 B).

Transverse section of root – The root shows well-developed anomalous secondary growth and has a thin periderm and a narrow cortex. Since the cortex is parenchymatous, it breaks separating the vascular cylinder. The secondary xylem is broken into four major fan-shaped radial segments, each of which is further cleaved into smaller lobes. Between the major lobes, broad radial files of parenchymatous cells form the cambium. In other regions, opposite to the xylem lobes, the cambium produces secondary phloem (Fig. 1C).

The secondary xylem consists of wide, angular, thin walled vessels and compact xylem fibres. The vessels are 100 to 200 µm wide. Secondary phloem consists of wide, horizontally oblong sieve elements with laterally placed companion cells. Phloem rays are distinct and radiate in the peripheral region. The sieve elements are 20 to 25 µm in diameter (Fig. 1D).

Powder characters

Stem – Non-lignified fibres are mostly seen and lignified fibres are occasionally observed (Figs. 2A and 2B). The fibres occur either singly or in groups of 2 - 8 and measure 460 - 570 - 650 µm in length. Rosette type of calcium oxalate crystals occur in two different sizes as small and large crystals measuring 18 - 21 and 22 - 36 µm in diameter respectively (Fig. 2C).

Root – The root powder is light brown in colour with a characteristic odour and slightly bitter taste. Vascular elements are seen with bordered pits. Non-lignified fibres are occasionally associated with vessels (Fig. 2D). Simple and compound starch grains are frequently seen. Small starch grains occur in both simple and compound forms and measure 5 - 12 - 17 µm in diameter. Large grains occur only in the simple form and measure 20 - 31 - 49 µm in diameter.

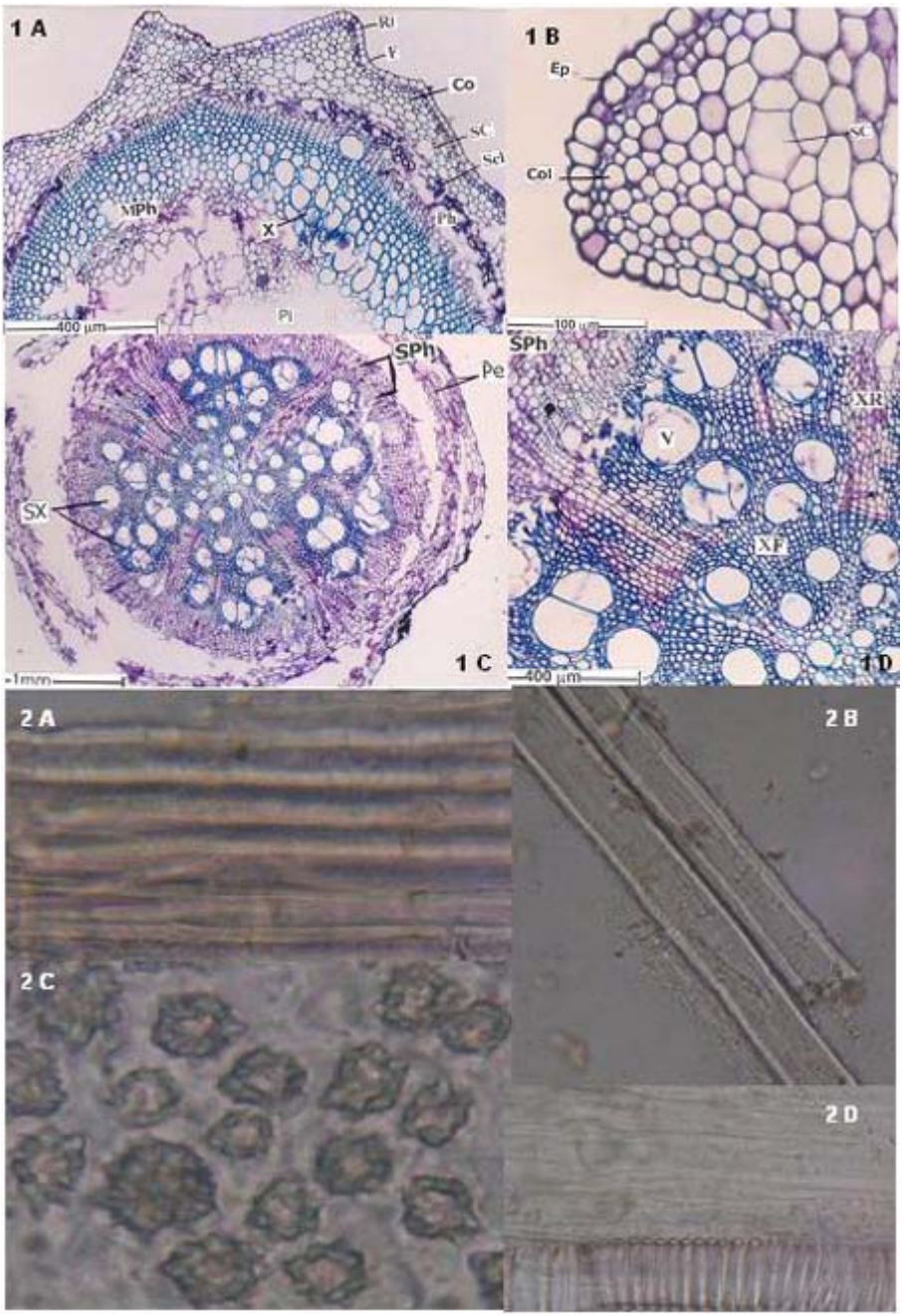

Fig. 1. A: T.S. of stem (**Ri**-Ridge; **F**-Farrow; **Co**-Cortex; **SC**-Secretary canal; **Scl**-Sclrenchyma; **MPh**-Medullary phloem; **X**-Xylem; **Ph**-Phloem; **Pi**-Pith), **B**: T.S of stem -a ridge portion enlarged (**Ep**-Epidermis; **Col**-Collenchyma; **SC**-Secretary canal), **C**: T.S. of root (**Pe**-Periderm; **SPh**-Secondary phloem; **SX**-Secondary xylem), **D**: T.S of secondary phloem and secondary xylem (**SPh**-Secondary phloem; **V**-Vessel; **XF**-Xylemfibre; **XR**-Xylem ray).

Fig. 2. Powder microscopy of stem and roots of *I. quamoclit.*
A: Lignified fibres in-group, **B**: Non-lignified fibres, **C**: Rosette ca. oxalate crystals, **D**: Fibres and vessel.

Preliminary phytochemical screening – Preliminary phytochemical screening revealed the presence of phytosterols, terpenes, phenolic compounds, carbohydrates, alkaloids and saponins (Tables 1 and 2).

Physico-chemical constants – Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and

Table 1. Preliminary phytochemical screening of the stem powder of *I. quamoclit*

Test	Hexane	Benzene	Chloroform	Acetone	Ethanol	Water
Alkaloids	–	–	+	+	+	–
Carbohydrates	–	–	–	–	+	+
Phytosterols	+	–	–	–	–	–
Terpenes	+	+	–	–	–	–
Fixed oils and fats	+	+	–	–	–	–
Saponins	–	–	–	–	+	+
Phenolic compounds and tannins	–	–	–	+	+	+
Flavonoids	–	–	–	+	+	–
Gums and Mucilages	–	–	–	–	–	+

+ Denotes the presence of the respective class of compounds

Table 2. Preliminary phytochemical screening of the root powder of *I. quamoclit*

Test	Hexane	Benzene	Chloroform	Acetone	Ethanol	Water
Alkaloids	–	+	+	–	–	–
Carbohydrates	–	–	–	–	+	+
Phytosterols	+	+	–	–	–	–
Terpenes	+	–	–	–	–	–
Fixed oils and fats	+	–	–	–	–	–
Saponins	–	–	–	–	–	–
Phenolic compounds and tannins	–	–	–	+	+	–
Flavonoids	–	–	–	–	–	–
Gums and Mucilages	–	–	–	–	–	–

+ Denotes the presence of the respective class of compounds

Table 3. Ash values of the stem and root powder of *I. quamoclit*

Parameters	Values % (w/w)	
	Stem powder	Root powder
Total ash	8.97	6.90
Acid insoluble ash	1.20	0.89
Water soluble ash	3.00	1.80
Sulphated ash	13.05	7.62

Table 4. Extractive values of the stem and root powder of *I. quamoclit*

Parameters	Values % (w/w)	
	Stem powder	Root powder
Water soluble extractive	5.90	1.20
Ethanol soluble extractive	7.50	1.99
Ether soluble extractive	0.83	0.95

Table 5. Fluorescence analysis of the stem and root powder of *I. quamoclit*

Treatment	Stem powder		Root powder	
	Day light	UV light (254 nm)	Day light	UV light (254 nm)
Powder as such	Light green	Light green	Light brown	Light brown
Powder + 1N NaOH (Aqueous)	Bright greenish yellow	Slightly brownish green	Slightly yellowish brown	Yellowish brown
Powder + 1N NaOH (Alcoholic)	Bright greenish yellow	Slightly yellowish brown	Yellowish brown	Yellowish brown
Powder + 1N HCl	Green	Green	Slightly reddish brown	Reddish brown
Powder + Ammonia	Yellowish green	Yellowish green	Dark brown	Fluorescent greenish brown
Powder + Iodine	Blackish green	Green	Dark brown	Dark brown
Powder + $FeCl_2$	Green	Dark green	Slightly greenish brown	Dark greenish brown
Powder + 1N H_2SO_4	Slightly brownish green	Brownish green	Brown	Dark brown
Powder + Acetic acid	Light green	Light green	Light brown	Dark brown
Powder + 1N HNo_3	Greenish brown	Greenish brown	Slightly reddish brown	Reddish brown

water soluble ash values (Table 3) and water soluble, alcohol soluble and ether soluble extractive values have been tabulated in Table 4. The extractive values are primarily useful for the determination of exhausted or adulterated drug. The results of fluorescence analysis of the drug powder are presented in Table 5.

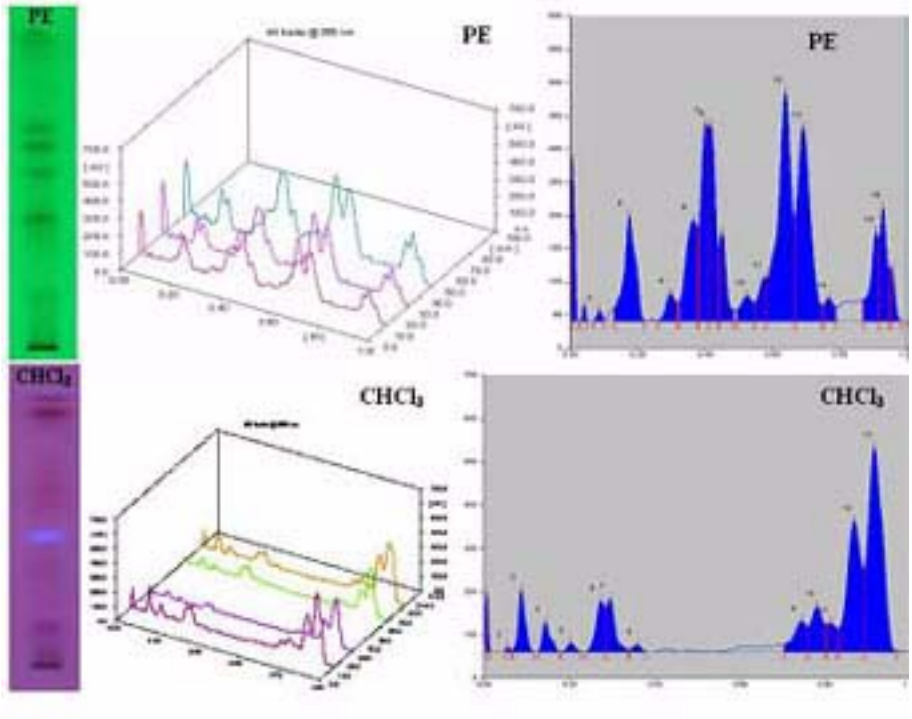

Fig. 3. HPTLC chromatogram of PE and $CHCl_3$ fractions of *I. quamoclit* stem (**PE**-Petroleum ether fraction; **$CHCl_3$**-Chloroform fraction).

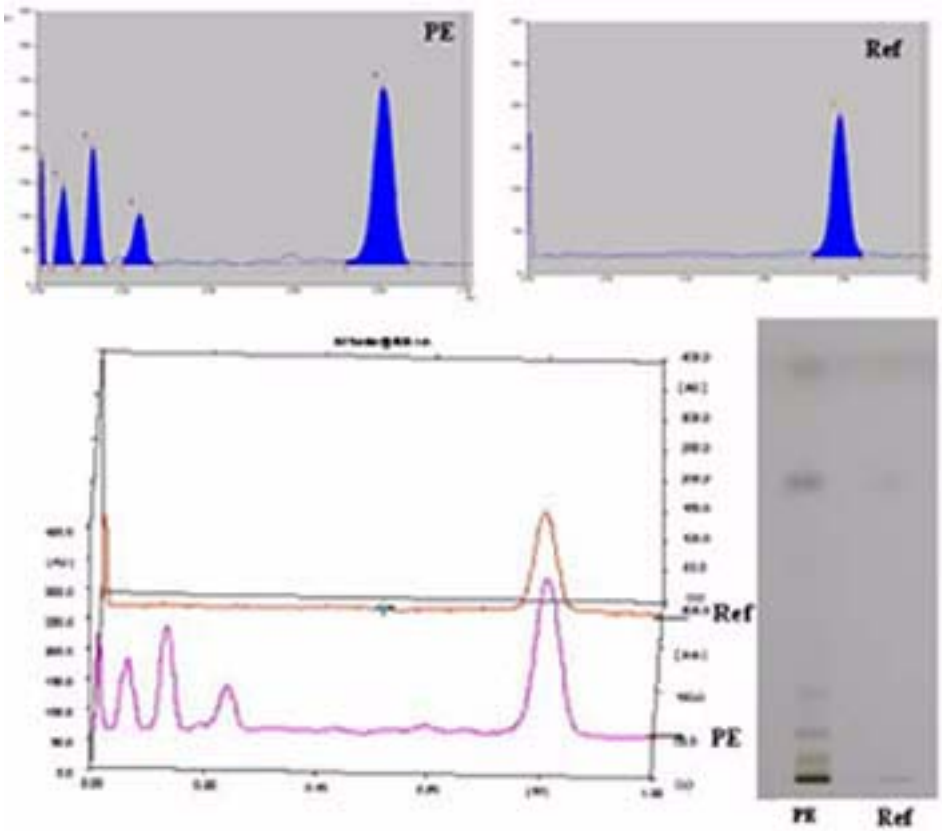

Fig. 4: HPTLC profile of PE fraction of *I. quamoclit* stem and Amyrin acetate reference (**PE**-Petroleum ether extract; **Ref**-Amyrin acetate reference).

HPTLC studies – A densitometric HPTLC analysis was performed for the development of characteristic finger print profile for petroleum ether and chloroform fractions of stems (Fig. 3), which may be used as markers for quality evaluation and standardization of the drug. Most of the compounds have shown maximum absorbance at 360 nm and 680 nm respectively for PE and $CHCl_3$ fractions. The bands in the sample were obtained at R_fs, 0.18, 0.30, 0.37, 0.40, 0.42, 0.45, 0.53, 0.58, 0.64, 0.69, 0.77, 0.91, 0.93, 0.95, (PE fraction) 0.14, 0.20, 0.27, 0.30, 0.36, 0.75, 0.78, 0.81, 0.87, 0.92 ($CHCl_3$ fraction), which can be used as identifying markers. Amyrin acetate (R_f– 0.81) content in the stem (Fig. 4) drug was found to be 0.11% w/w.

Conclusion

The present study on pharmacognostical evaluation of the stem and root of *I. quamoclit* will provide useful information for its identification. Macro and micro morphological standards discussed here can be considered as identifying parameters to substantiate and authenticate the drug.

Acknowledgements

The authors are thankful to Manipal Academy of Higher Education, Manipal for providing the facilities to carry out the study and Manipal Foundation for awarding Faculty Study Fellowship Grant.

References

Agarwal, V.S., *Drug plants of India*, Kalyani Publishers, New Delhi, I[st] ed., Vol. I, pp. 410-412, 1997.

Brain, K.R. and Turner, T.D., *The Practical Evaluation of Phytopharmaceuticals*, Wright-Scientechnica, Bristol, pp. 4-9, 1975a.

Brain, K.R. and Turner, T.D., *The Practical Evaluation of Phytopharmaceuticals*, Wright-Scientechnica, Bristol, pp. 36-45, 1975b.

Chase, C.R. and Pratt, R.J., Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *J. Am. Pharmacol. Assoc* **38**, 32 (1949).

Deepa, M.A., Narmatha Bai, V., and Basker, S., Antifungal properties of *pseudarthria viscida*. *Fitoterapia* **75**(6), 581-584 (2004).

Harborne, J.B., Methods of extraction and isolation. In: *Phytochemical Methods*, Chapman & Hall, London, pp. 60-66, 1998.

Indian Pharmacopoeia, 4[th] edn., Vol. II, Government of India, Ministry of Health and Welfare, Controller of Publications, New Delhi, pp. A53-A54, 1996.

Johansen, D.A., *Plant Microtechnique*, McGraw Hill, New York, pp. 182, 1940.

Kirtikar, K.R. and Basu, B.D., *Indian Medicinal plants*, Bishen Singh and Mahendra Pal Singh, Dehradun: 2[nd] ed., Vol. III, pp. 1712-1713, 1991.

Kokate, C.K., *Practical Pharmacognosy*, 1[st] ed., Vallabh Prakashan, New Delhi, pp. 15-30, 1986a.

Kokate, C.K., *Practical Pharmacognosy*, 1[st] ed., Vallabh Prakashan, New Delhi, pp. 111, 1986b.

Kokoski, J., Kokoski, R., and Slama, F.J., Fluorescence of powdered vegetable drugs under ultraviolet radiation, *J. Am. Pharmacol. Assoc* **47**, 715 (1958).

Loustalot, A.J. and Pagan, C., Local "fever" plants tested for presence of alkaloids, *EL Crisol (Puerto Rico)* **35**, 3 (1949).

The Wealth of India, *Raw Materials*, New Delhi: CSIR, NISCOM, Vol. V, pp. 252-253, 1998.

WHO/PHARM/92.559/rev.1., *Quality Control Methods for Medicinal Plant Materials*, Organisation Mondiale De La Sante, Geneva, pp. 9, 22-34, 1992.

Yoganarasimhan, S.N., *Medicinal Plants of India*. Tamilnadu. Bangalore, India: Regional Research Institute (Ay.), Vol. II, pp. 262, 2000.

(Accepted July 16, 2007)