Pharmacogn J. 2017; 9(5):657-662
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Microscopical Evaluation, Phytochemical Analysis and HPTLC Fingerprinting of Tuber of *Actinoscirpus grossus* (L.f.) *Goetgh. & D.A.Simpson*

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History
- Submission Date: 07-05-2017;
- Review completed: 27-05-2017;
- Accepted Date: 18-06-2017

DOI : 10.5530/pj.2017.5.104

Article Available online
http://www.phcogj.com/v9/i5

ABSTRACT
Actinoscirpus grossus (L.f.) Goetgh. & D.A.Simpson (Cyperaceae), is a Perennial with long stolons and rhizomes ending in small tubers. It is popularly known as *Kasheruk* in Sanskrit. The plant is traditionally used as anti-diarrheal, anti-emetic, and tonic to the liver. In order to do the detail standardization of plant macro-microscopical observation, phytochemical analysis and HPTLC Finger printing of tuber was performed according to pharmacopoeia procedure. Microscopic analysis has showed thick-walled polygonal epidermal cells of young root stalk in surface view, elongated phloem parenchyma filled with starch grains, spiral to annular vessel fragments and simple starch grains scattered all over the powder. Phytochemical analysis showed presence of carbohydrate, coumarins, flavanoids, steroid, tannin, and terpenoid. Ethanol extract of plant were fingerprinted in toluene: ethyl acetate (7:3). The developed plates were visualized in UV 254, 366, and then derivatised with vanillin sulphuric acid and scanned under UV 254 and 366 nm. These specific identities will be useful in identification and authentication of the raw drug.
Key words: Ethanol extract, HPTLC, Pharmacognosy, Phytochemical analysis, Quality control, Standardization.

INTRODUCTION

The plant *Actinoscirpus grossus* (L.f.) Goetgh. & D.A.Simpson of Cyperaceae family is popularly known as *Kasheruk* mentioned in dravyaguna.[1] *A. grossus* is one of traditional herbs which claims to be having many useful properties anti diarrheal, anti-emetic, non-specific anti spasmodic, progesterone like activity, and used in digestive disorders. The root is slightly sweet, cooling, laxative, tonic to the liver, diuretic, useful in burning sensations, vomiting, diarrhea[2,3] and has astringent property.[4] The tuber of *A. grossus* was also used traditionally as hepatoprotective agent. *A. grossus* is principal weed of four South East Asian Countries occuring in swampy and inundated places, pools, ditches and marshses.[5] There are many families of phytochemicals helping a human body in a variety of ways. Traditional folk remedies from plants have always guided scientists to search for new medications in order to maintain healthy life for humans.[6] Before starting any trial on a drug it has to undergo detailed standardization, chemical analysis and pharmacological property evaluation. Pharmacognostical study is the initial step to confirm the identity and to assess the quality and purity of crude drug.[7] Due to many reasons such as similar morphological features, presences of similar active principles etc, medicinal plant materials were being adulterated. Therapeutic activity of herbal products is affected badly by practice of substitution and adulteration, therefore systematic identification of drugs and their substitutes is an essential step while producing standardized herbal products. Standardization of herbal drug is not an easy task as numerous factors influence the bio efficacy and reproducible therapeutic effect in order to obtain quality oriented herbal products.[8] In the present study standardization of tuber extract *A. grossus* was performed. Preliminary phytochemical study, powder microscopy and HPTLC of tuber extract of plant are documented.

MATERIALS AND METHODS

Plant materials

The authenticated sample of plant tuber powder of *A. grossus* was purchased from Vaidya Hukam Chand Arogyadham Gharaunda, Haryana India. The voucher specimen (No. 495/14101822) was deposited at the Pharmacognosy Laboratory of S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi for future reference. For powder microscopy, the sample was sift through mesh 60; the powder was stored in glass vial for the phytochemical examination and HPTLC study.

Cite this article : Savin GC, Holla R, Shankara S, Sunil Kumar KN, Ravi M. Title-Microscopical Evaluation, Phytochemical Analysis and HPTLC Fingerprinting of Tuber of *Actinoscirpus grossus* (L.f.)Goetgh. & D.A.Simpson. Pharmacog J. 2017;9(5):657-62.

Powder microscopy

A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerin. Slides observed under microscope and diagnostic characters were observed and photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.[9]

Preliminary phytochemical analysis

Preliminary phytochemical investigation was done with the coarse powder to detect the presence of carbohydrate, coumarins, flavonoids, steroid, tannin, and terpenoid in ethanolic extract.[10]

HPTLC

1g of powder was extracted with 10 ml of ethanol, 4, 8 and 12 µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7:3). The developed plates were visualized in UV 254, 366, and then derivatised with vanillin sulphuric acid and scanned under UV 254 and 366 nm and colour of the spots and densitometric scan were recorded.[11-14]

RESULTS

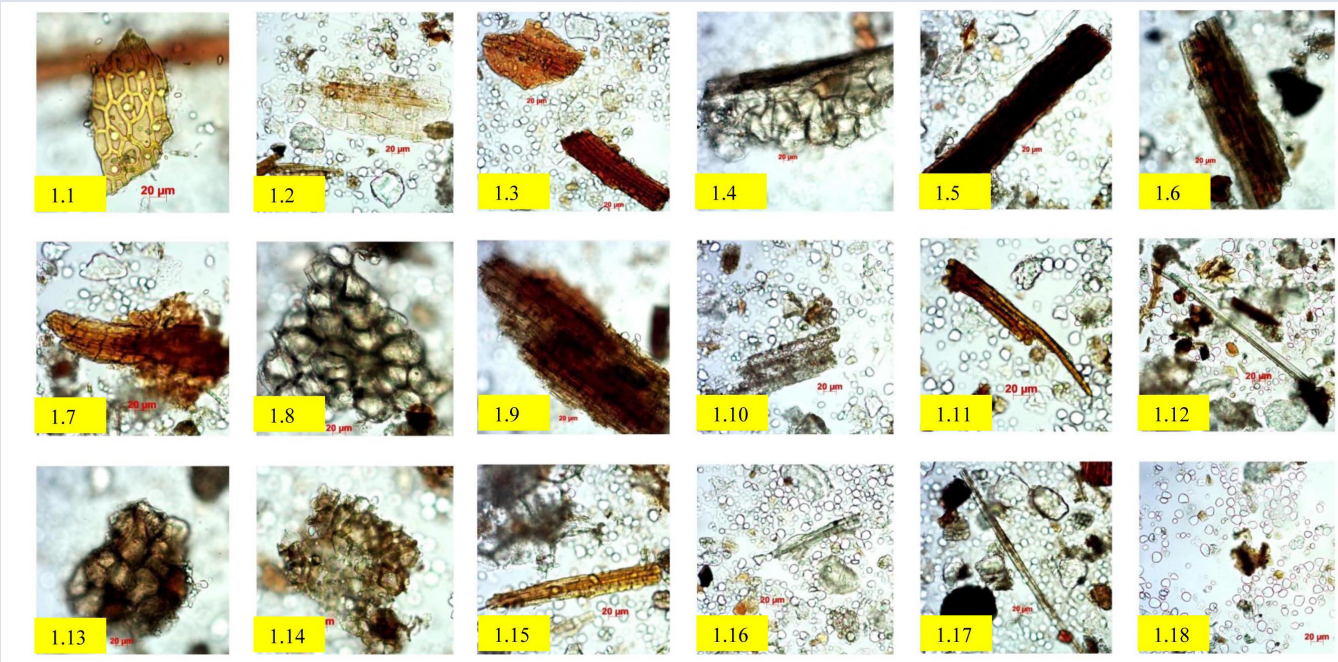
Powder microscopy study of tuber powder of *A. grossus* showed characters such as thick-walled polygonal epidermal cells of young root stalk in surface view, cells of cork in few rows in surface view, longitudinally cut cork cells in few layers, parenchyma as such or attached with thin-walled simple and pitted fibres, thick and thin walled parenchyma with or without yellowish brown contents, elongated phloem parenchyma filled with starch grains, spiral to annular vessel fragments and simple starch grains scattered all over the powder [Figure 1].

HPTLC photo documentation of alcoholic extract of *A. grossus* showed 7, 13 and 14 spots with characteristics R_f value and colors under short UV, long UV and white light after derivatisation respectively [Table 1]. Spots with R_f value 0.27, 0.36 and 0.81 were detected in all three-detection method though in different colors. HPTLC densitometric scan at 254nm showed maximum number of detectable spots counting to 11 with different percentage area. HPTLC with photo documentation and densitometric scan can be used as finger print of chemical component present in ethanol extract of *A. grossus* [Figure 2,3].

Powdered tuber extract of plant *A. grossus* showed presence of carbohydrate, coumarins, flavonoids, steroid, tannin, and terpenoid [Table 2].

DISCUSSION

The plant *A. grossus* is a principal weed of four South East Asian countries. It is well known herb which is used in Indian System of medicine. Medicinal plant materials are being adulterated due to many reasons. The practice of substitution and adulteration will badly affect the therapeutic activity of herbal products. Microscopical analysis of plants is invaluable for assuring the identity of the material and as initial screening test for impurities.[15]Authentication and quality assessment of herbal materials deals with the pharmacognosy that is based on macroscopic and microscopic features. A big quantum of research works in the area of authentication of the correct plant source has been undertaken to provide means



1.1 Epidermis in surface view; 1.2 Parenchyma with thin-walled fibre; 1.3 Parenchyma with contents; 1.4 Pith parenchyma; 1.5 and 1.6 Parenchyma with contents; 1.7 Thick-walled parenchyma; 1.8 Cells of cork in surface view; 1.9 Longitudinally cut cork cells; 1.10 Phloem parenchyma with starch; 1.11 Elongated parenchyma with contents; 1.12 Thin-walled fibres; 1.13 Parenchyma with contents; 1.14 Cortical parenchyma; 1.15 Thin-walled fibres and parenchyma; 1.16 Vessel fragments and starch grains; 1.17 Pitted fibres; 1.18 Simple starch grains.

Figure 1: Powder microscopy of tuber of *Actinoscirpus grossus* (L.f.) Goetgh.&D.A.Simpson

Table 1: R_f values of ethanol extract of tuber of *Actinoscirpus grossus* (L.f.) Goetgh. and D.A. Simpson at 254 nm (12 µl)

Under short UV	Under long UV	Under white light after derivatisation
-	0.03(F L Violet)	-
-	0.06(F L Violet)	0.06(Violet)
-	0.09(F L Violet)	-
-	-	0.12(Violet)
-	0.14(F L Violet)	-
0.16(L Green)	-	-
-	0.18(F L Violet)	-
-	-	0.21 (L Violet)
0.27(L Green)	0.27(F L Violet)	0.27(L Violet)
-	-	0.32(L Violet)
0.36(L Green)	0.36(F L Violet)	0.36(L Violet)
-	-	0.38(L Violet)
-	0.40(F L Violet)	-
-	0.44(F L Violet)	0.44(Violet)
0.46(L Green)	-	0.46(L Violet)
-	-	0.52(L Violet)
-	0.54(F L Violet)	-
0.56(L Green)	-	-
-	0.60(F L Violet)	0.60(L Violet)
-	-	0.67(L Violet)
0.81(L Green)	0.81(F Violet)	0.81(L Violet)
0.86(Green)	-	-
-	-	0.92(L Violet)
-	0.95(F L Violet)	-

L-Light, D-Dark, F-Fluorescence

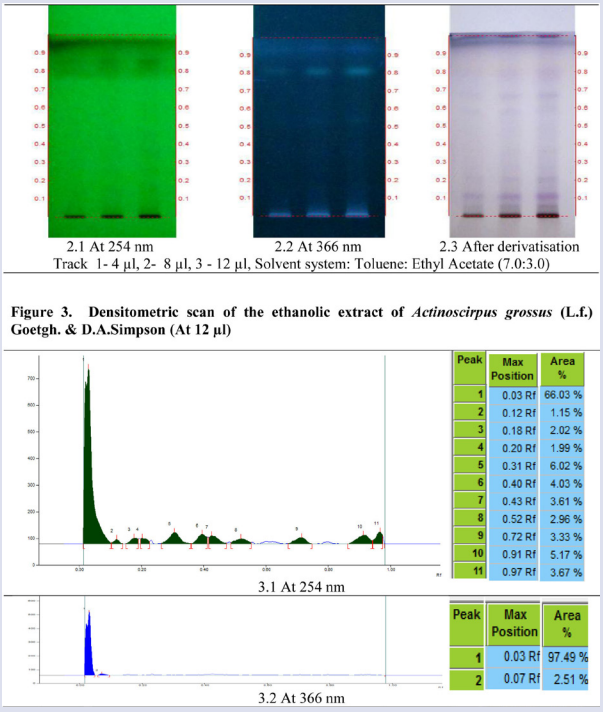


Figure 2: Photo documentation of ethanolic extract of *Actinoscirpus grossus* (L.f.) Goetgh.&D.A. Simpson

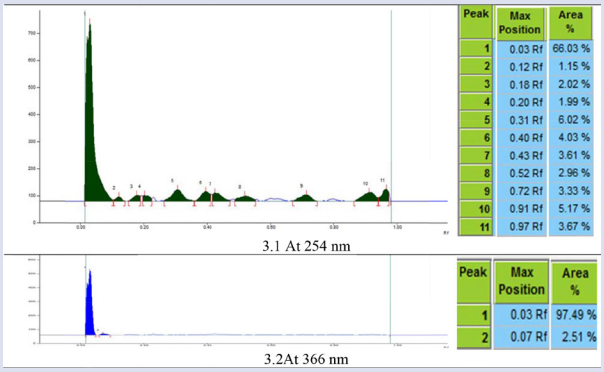


Figure 3: Densitometric scan of the ethanolic extract of tuber of *Actinoscirpus grossus* (L.f.) Goetgh.&D.A. Simpson (12 µl).

of differentiation among many available plant sources.[16-18] Sophisticated modern research tools for evaluation of the plant drugs are available but microscopic method is still one of the simplest and cheapest methods to establish the correct identity of the source of material.[19] According to the world health organization (WHO) the macroscopic and microscopic description of a plant is the first step to establish the identity before any tests are undertaken.

Preliminary phytochemical analysis showed presence of carbohydrate, coumarins, flavonoids, steroid, tannin, and terpenoid which may help in the hepatoprotective activity of plant.

Table 2: Preliminary phytochemical analysis ethanol extract of tuber of *Actinoscirpus grossus* (L.f.) Goetgh. D.A.Simpson

Tests	Colour if positive	*Acotinoscirpus grossus*	Results
Alkaloids			
Dragendrof's test	Orange precipitate	Brown Colour Solution	
Wagners test	Red precipitate	Brown Colour Solution	Absent
Mayers test	Dull white precipitate	Light yellow Colour	
Hagers test	Yellow precipitate	Light yellow Colour	
Steroids			
Liebermann- buchard test	Bluish green	Green colour	Present
Salkowski test	Bluish red to cherry red	Red colour at junction	
Carbohydrate			
Molish test	Violet ring	Violet ring	
Fehlings test	Brick red precipitate	Brick red precipitate	Present
Benedicts test	Red precipitate	Red precipitate	
Tannin			
With $FeCl_3$	Dark blue or green or brown	Dark Green colour.	Present
Flavanoids			
Shinoda's test	Red to pink	Pink Colour	Present
Saponins			
With $NaHCO_3$	Stable froth	No froth	Absent
Triterpenoids			
Tin and thionyl chloride test	Pink	Pink Colour	Present
Coumarins			
With 2 N NaOH	Yellow	Yellow Colour Solution	Present
Phenols			
With alcoholic ferric chloride	Blue to blue black, brown	Dark Green Colour	Absent
Carboxylic acid			
With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence	Absent
Resin			
With aqueous acetone	Turbidity	No Turbidity	Absent
Quinone			
5% NaOH	Pink/purple/red	Dark Green Colour	Absent

HPTLC is one of the sophisticated instrumental techniques for qualitative and quantitative analysis of the herbs and herbal drugs. HPTLC serves as quality assessment tool which helps in identification of variation in chemical composition in plants. HPTLC fingerprinting shows different R_f values can be used as quality indicating finger printing for *A. grossus* in the dried form. High-performance thin-layer chromatography (HPTLC) is a form of thin-layer chromatography (TLC) that provides superior separation using optimized coating material, novel procedures for mobile-phase feeding, layer conditioning, and improved sample application. It promotes for higher separation efficiencies, shorter analysis time, lower amounts of mobile phase, and efficient data acquisition and processing.[20]Among the modern analytical tools HPTLC is a powerful analytical method equally suitable for qualitative analytical tasks HPTLC produces visible chromatogram complex information about the entire sample and multiple samples are seen simultaneously, so that reference and test samples can be compared for identification. The analysis and quality control of herbal medicines are moving a step ahead towards an integrative and comprehensive direction, to tackle the complex nature of herbal medicines. High-performance thin layer chromatography (HPTLC) is one of the sophisticated instrumental techniques for qualitative and quantitative analysis of the herbs and herbal drugs.[21,22] Marker compound means chemical constituents within a medicinal plant that can be used to verify its potency or identity. For sometimes, the marker compounds may be described as active ingredients or chemicals that confirm the correct botanical identity of the starting material. It is very difficult to identify correct marker compounds for all traditional medicines, because some medicines have unknown active constituents and others have multiple active constituents. By using chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents is not the same for different samples of drug.[23-25]

One of the drawbacks of herbal medicine is lack of standardization and quality control profiles of correct identification of species. The mistaken substitution and misclassification of species is real danger in preparation and administration of herbal medicine. The features available in the powder form of the given specimens are useful to distinguish the samples even in a mixture form.[26,27]

CONCLUSION

The given samples have been tested as per standard testing protocol. The tuber powder microscopical characters were examined and reported. The extract was tested positive for carbohydrate, coumarins, flavonoids, steroid, tannin, and terpenoid. HPTLC photo documentation, R_f values and densitometric scan at 254 nm, 366 nm and after derivatisation has been developed and reported.

ACKNOWLEDGEMENT

The first author is grateful to KSHEMA, NITTE University Deralakatte for the opportunity and Dr. K.V. Chidananada, President, AOLE & Medical Director KVG Medical college, Sullia for support.

CONFLICT OF INTEREST

No conflict of interest are declared.

ABBREVIATIONS USED

A. grossus: *Actinoscirpus grossus* (L.f.) Goetgh. & D.A.Simpson; **HPTLC:** High performance thin layer chromatography.

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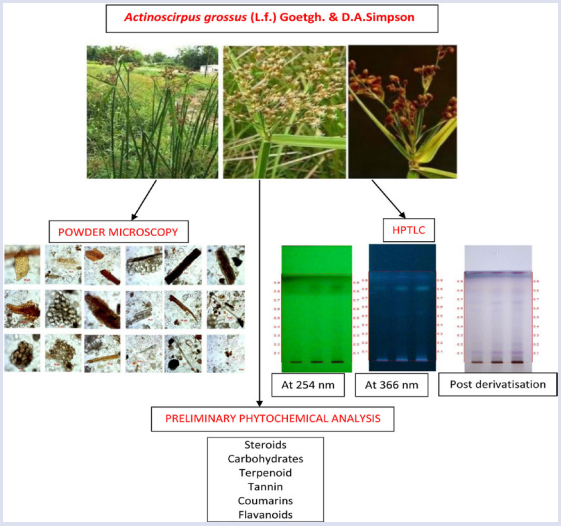
GRAPHICAL ABSTRACT



HIGHLIGHTS OF PAPER

- Powder of tuber of *Actinoscirpus grossus* (L.f.) Goetgh.&D.A.Simpson standardized by microscopy
- Ethanol extract of tuber of *Actinoscirpus grossus* (L.f.) Goetgh.&D.A.Simpson subjected for preliminary phytochemical examination
- Ethanol extract of tuber of *Actinoscirpus grossus* (L.f.) Goetgh.&D.A.Simpson fingerprinted by HPTLC
- Quality standards for tuber powder of *Actinoscirpus grossus* (L.f.)Goetgh.&D.A.Simpson proposed for routine testing and analysis of authenticity of the raw drug.

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Cite this article : Savin GC, Holla R, Shankara S, Sunil NKK, Mundugaru R. Title-Microscopical Evaluation, Phytochemical Analysis and HPTLC Fingerprinting of Tuber of *Actinoscirpus grossus* (l.f.) GOETGH. & D.A.Simpson. Pharmacog J. 2017;9(5):657-62.