Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES OF TWO APHRODISIAC PLANTS – *CHLOROPHYTUM BORIVILIANUM* SANTAPAU & FERNANDES AND *CHLOROPHYTUM TUBEROSUM* (ROXB.) BAKER

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Received: 01 Aug 2013, Revised and Accepted: 02 Sep 2013

ABSTRACT

Objective: *Chlorophytum borivilianum* and *Chlorophytum tuberosum* are economically and medicinally very important species in India. Both of the species look similar in nature and contain same type of saponin. So, there remains ample scopes to merchandise the plant parts or products of the second species in the name of the former, which although does not matter regarding the quality of product, but certainly do so in the parlance of economy. In the present study an effort has been made to work out the identifying characters and detail comparative accounts of both species for ready identification of entire plant, live or in herbarium and also the discrimination of drugs made from tubers of two species.

Methods: Morphology and anatomical studies were carried out with the help of compound light microscope. Saponin was characterized through the HPTLC and FTIR analyses. Other phytochemical studies, pertinent to evaluation of drug quality, were also carried out.

Results: Flower and tuber features of two species showed little differences. Anatomical investigation revealed greater number of xylem strands in *C. tuberosum* than the other. Organoleptic observations of both species expressed differences. FTIR and HPTLC analyses showed the presence of similar type of saponin in both of them.

Conclusion: Consanguinity of two species makes delimitation and ready recognition of them harder. Similarities and dissimilarities between two species revealed in the present study in different forms of the plants, live or herbarium, entire or part or in powder form will succor well in discriminating them. Commercially available tuber powder of both the species can be discriminated with the aid of this study and the adulterants thereof can be checked.

Keywords: *Chlorophytum borivilianum*, *Chlorophytum tuberosum*, Saponin, FTIR, Drug identification, Pharmacognosy.

INTRODUCTION

The genus *Chlorophytum* Ker-Gwal belongs to the family Asparagaceae. Two species of this genus *Chlorophytum borivilianum* and *C. tuberosum* are popularly known as 'Safed Musli' and both of them have medicinal importance due to the presence of saponin. Species of the genus are distributed throughout the tropical and sub-tropical regions of the world and overall 256 species have been recorded, whereas only 13 species are being reported to occur in India [1, 2]. Two congeneric species *C. borivilianum* and *C. tuberosum* are morphologically very analogous [3], though, the former species is considered to be commercially important due to the occurrence of more saponin content than the latter. Both of the species are used as sources of main ingredient of medicines of various forms like, tonic, massage oil, capsule, powder etc. by different companies of medicine industry for the remedy of various maladies. As a result, it appears to have every possibility of the aphrodisiac drug prepared with *C. tuberosum* be claimed of *C. borivilianum*. Although the quality of drug hardly differs with this adulteration, the cost of the product will matter due to *C. borivilianum* being costlier than the other. In West Bengal, *C. tuberosum* is used abundantly as 'safed musli' by the village based traditional medical practitioners in this state of India. The gross morphological similarities confuse the identification of two species of *Chlorophytum*. A close study on morphology revealed that the plant height is slightly different for two species; *C. tuberosum* being slightly taller than *C. borivilianum*. Leaf margin of *C. borivilianum* is plane, while in *C. tuberosum* it is undulated. Plant anatomy plays an important role in pharmacognostic study to discriminate desirable species from the spurious ones. Anatomical features of medicinal plants for the authenticity and quality control of the drugs are very much useful [4, 5]. According to World Health Organization, the macro and microscopic studies are required for the purpose of identification of any medicinal plant, prior to performing other tests with the plant products, for the purpose of controlling the purity of prepared medicine [6]. During last two decades, the pharmaceutical industry has made massive investments on pharmacological, clinical and chemical researches all over the world in an effort to launch more and more potent plant drugs [7]. In view of such importance critical characterization of two species in respect of tuber anatomy and micro-morphology of powder drugs has been presented in this study, which may be effective in rightly identifying the plant species and their products even; any adulteration in the drugs made up thereof can also be detected.

MATERIALS AND METHODS

Collection of Plant Materials

Chlorophytum borivilianum Santapau and Fernandes was collected from Jeevan Herbs, Sagar, Madhya Pradesh and medicinal plants' garden of Directorate of Forests, Government of West Bengal at Paschim Medinipur. *Chlorophytum tuberosum* (Roxb.) Baker was procured from the different wild forest areas of West Bengal.

Morphological and Anatomical Study

Fresh plant parts were observed thoroughly under a dissecting microscope. The observed physical characters of different plant parts were verified from the book 'Bengal Plants' authored by David Prain [8] and studying the herbaria of Central National Herbarium, Howrah. The voucher specimens have been deposited at the herbarium of Department of Botany and Forestry, Vidyasagar University, Midnapore, West Bengal.

Thin sections (20 µm) of fresh leaves and tubers of both of the species were made and observed under the Leica DM1000 microscope following Iyengar and Nayak [9].

Microscopy of powder

Freshly collected tubers were dried on sunlight and homogenized with mortar and pestle to make fine powder. The powder was smeared on slides and observed under light microscope (*Leica DM1000*).

Physico-chemical Study

Organoleptic study of tuber powder, analysis of physico-chemical parameters, such as responses of the powder under influence of

different chemicals observed with visible and fluorescence light (254 nm and 366 nm) and analysis of total ash value, acid-insoluble ash and water soluble ash were determined according to the standard procedures [6,7,10,11,12].

Qualitative and Quantitative Study

Quantity and quality of the active principle i.e. saponin was measured with HPTLC (Camag) and FTIR spectroscopy (Model - Perkin Elmer, version 10.03.07) using KBr pellets was observed at different wavelengths in FTIR spectrophotometer.

Phyto-chemical Screening

Preliminary phytochemical screenings for the detection of various active chemical constituents were carried out following Harborne, Laha and others [11, 12, 13, and 14].

RESULT

Morphology

The detail morphological study revealed that the plant height has been found to be slightly different for two species; *C. tuberosum* being slightly taller than *C. borivilianum*. Leaf margin of *C. borivilianum* is plane, while in *C. tuberosum* it is undulated (fig 1a & b). Only the floral morphology has been found to be quite apt to distinguish two species right on the field. Inflorescence is raceme for both of them. Flowers are bisexual, white, perianth six in number, incurved in *C. tuberosum* and recurved outwardly in *C. borivilianum*. Androecia are six, stamens of *C. tuberosum* are not longer than the style, but in case of *C. borivilianum* it is opposite (fig 2a & b). Three lobed gynoecium and axillary placentation are common for two species. Three lobed capsules with flattened black seeds are same for both of them. Cylindrical and fascicular tubers in *C. borivilianum* are tapering towards the free ends and in *C. tuberosum* they are tapering towards both ends (fig 3a & b).

Anatomical studies and microscopy of powder

Transverse sections of leaf of both of the species showed similar characters in having closed collateral vascular bundles (fig 5) and diacytic type of stomata (fig 6). Transverse section of tubers showed ~~a~~ little difference by the presence of saponin in the cortex cell of *C. borivilianum,* while confined to epidermal layer in *C. tuberosum*. Contrast was noted in the number of xylem strand too, which was 9 - 10 in *C. borivilianum* but 11 - 15 in *C. tuberosum*. Rhaphides were found to occur profusely in the cortex cells and pith cells of *C. borivilianum*, but it is only scanty in the cortex cells and totally absent in the pith cells of *C. tuberosum* (fig 8).

Microscopic studies of powdered tuber of both of the species showed scalariform and spirally thickened vessels, xylem fibers, and parenchyma cells (fig7 & 8). *C. borivilianum* showed acicular rhaphides, whereas, it is blunt at one end and sharp at the other in *C. tuberosum*. A few stone cells were found to have only *C. borivilianum* powder (fig 7).

Stereomicroscopic study of dry saponin powders extracted from both the species has shown resemblance (fig 9a. & b.).

Organoleptic and Physico-chemical Studies

Organoleptic studies revealed difference between two species (table 1). *C. tuberosum* showed highest amount of total ash, acid and water soluble ash than *C. borivilianum* (table 2).

Qualitative and Quantitative Studies

FTIR spectrum exhibited absorption in the range from 3409.58 cm^{-1} to 1716.42 cm^{-1} (fig 11). IR spectrum exhibited a long and sharp peak in the range of 3409.58 to 3404.80 cm^{-1} for hydroxyl group. For carboxylic acids group IR indicated a sharp peak at 2361.34 cm^{-1} to 2357 cm^{-1} and peak range 2926.89 cm^{-1} to 2925.46 cm^{-1} clearly verified the presence of alkenes. Presence of keto group was indicated by the peak 1719.74 cm^{-1} to 1716.02 cm^{-1} (fig 11). HPTLC of saponin revealed to contain more amount of saponin *C. borivilianum* than *C. tuberosum* (fig 10).

Phyto-chemical Screening

Both the species have same type of secondary active components. Some active components like flavonoids, phenols, anthraquinone glycosides were noted to be completely absent in both of the species (Table 6).

Fig. 1a) Habit of *Chlorophytum borivilianum* b) Habit of *Chlorophytum tuberosum*

Fig. 3a) Tubers of *C. borivilianum* b) Tubers of *C. tuberosum*

Fig. 2a) Flowers of *C. borivilianum* b) Flowers of *C. tuberosum*

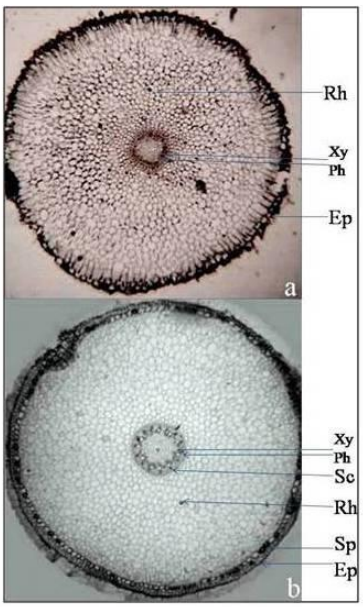

Fig. 4: Transvers section of tuber. a) *C. borivilianum* b*) C. tuberosum* (Rh-Raphides, Xy-Xylem, Ph-Phloem, Ep-Epidermis, Sc-Schlerides, Sp-Saponin).

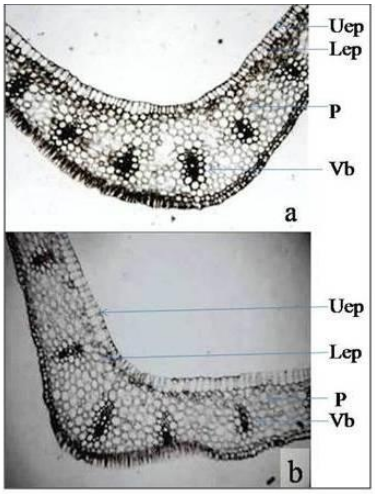

Fig. 5: Transverse section of leaf. a) *C. borivilianum* b) *C. tuberosum* (Uep-Upper Epidermis, Lep-Lower epidermis, P-Parenchyma cells, Vb-Vascular bundle).

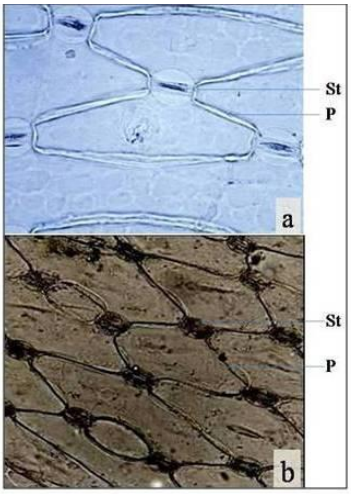

Fig. 6: a) Stomata of *C. borivilianum* b) Stomach of *C. tuberosum* (St-Stomata,P-Parenchyma cells)

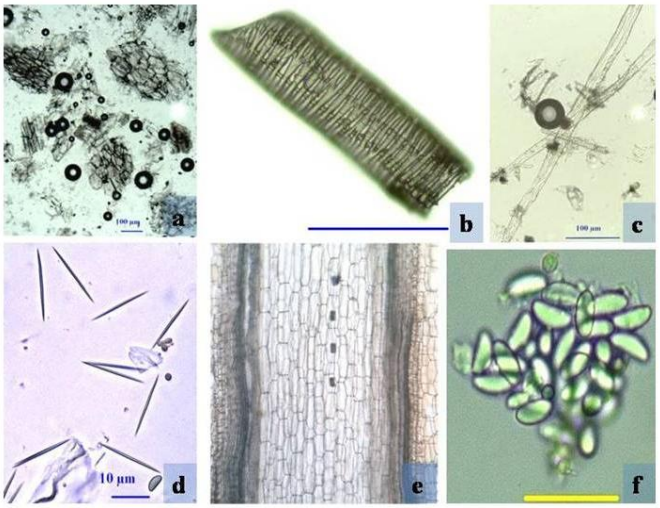

Fig. 7: Power microscopic study of *C. borivilianum* a) Presence of saponin, parenchyma cells b) Spiral arrangement of tracheids c) Xylem fibre d) acicular rhaphides e) Longitudinal section of tuber showing the presence of rhaphide bundle in pith cells f) Stone cells.

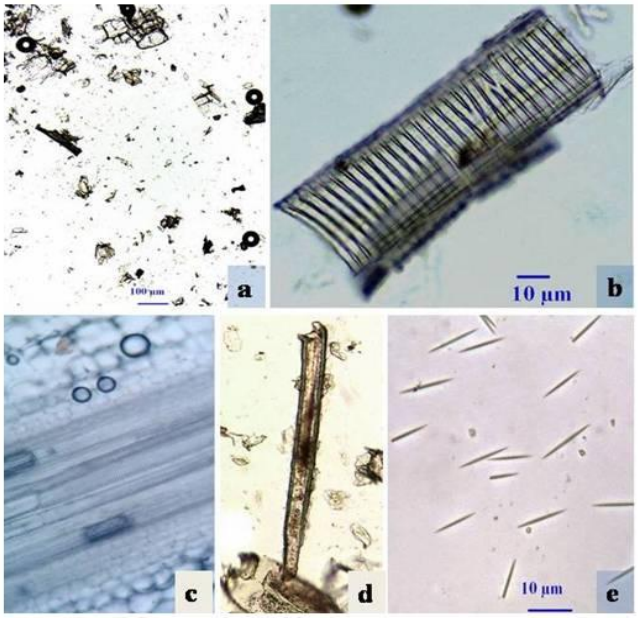

Fig. 7: Power microscopic study of *C. tuberosum* a) Presence of saponin, parenchyma cells b) Spiral tracheids c) Longitudinal section of tuber showing the presence of rhaphide bundle in pith cells d) Xylem fibre e) acicular and blunt end rhaphides.

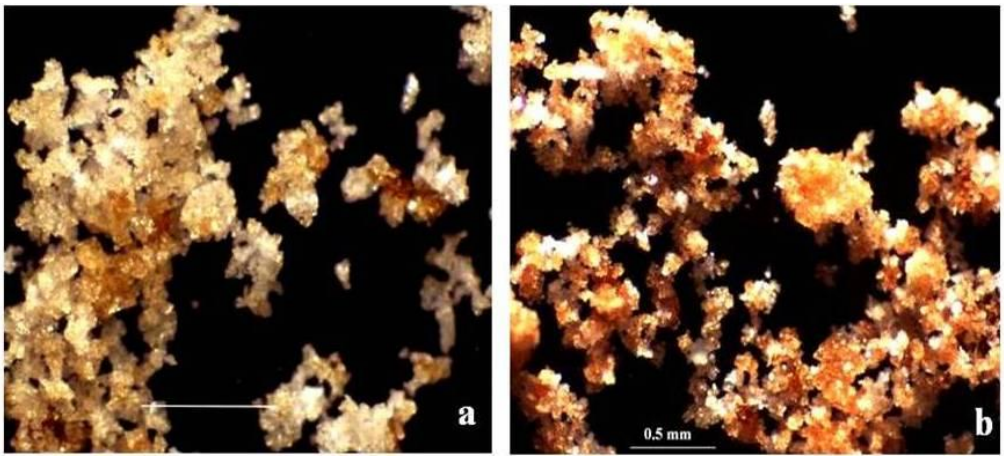

Fig. 9: Extracted saponin powder under the stereo microscope. a) Saponin of *C. borivilianum* b) Saponin of *C. tuberosum*

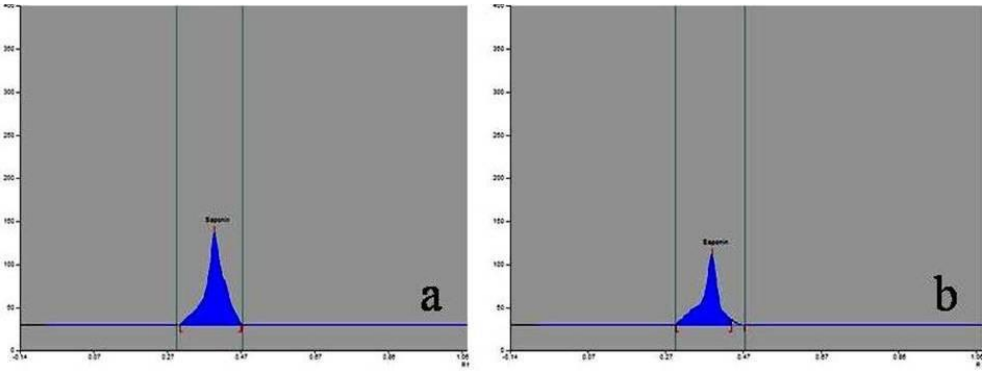

Fig. 10: Saponin peaks in HPTLC. a) *C. borivilianum* and b) C. *tuberosum*.

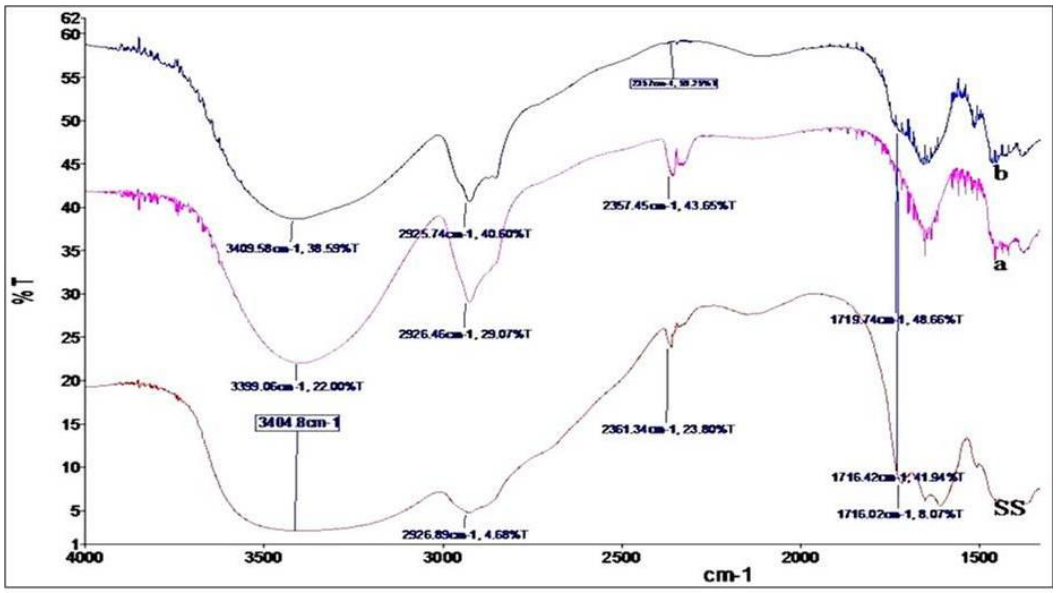

Fig. 11: FTIR graph of saponin (SS) Standard saponin, a) *C. borivilianum* and b) *C. tuberosum*.

Table 1: Organoleptic study of *C. borivilianum* and *C. tuberosum*

Character	*C. borivilianum*	*C. tuberosum*
Colour	Creamy white	Creamy Brown
Odour	Milk-like odour	Old dry plant tissue
Test	Tasteless and Psyllium husk like	Little bit bitter
Texture	Smooth and sticky	Rough

Table 2: Physico – chemical parameters of the species

Character	*C. borivilianum*	*C. tuberosum*
Total ash content	12.20%	13.10%
Acid insoluble ash	03.80%	04.50%
Water soluble ash	07.70%	08.20%

Table 3: Tuber anatomy of both the species

Anatomy of Tuber	*C. borivilianum*	*C. tuberosum*
Epidermis	Withered away at places	Multilayered with hairs
Hypodermis	Multi-layred	Single layered
Type of vascular bundle	Radial polyarch	Radial polyarch
Endodermis	Single layered	Single layered
Pericycle	Uniseriate	Uniseriate
Xylem strand	**09 – 10**	**11 – 15**
Raphides	Profuse	Scanty
Schlerides	Rarely present	Plenty

Table 4: Microscopic study of tuber powder of two species

Elemenmts	*C. borivilianum*	*C. tuberosum*
Tracheid	Spiral	Spiral
Trachea	Reticulate and Scalariform	Scalariform
Xylem Fiber	Long and pitted	Long and pitted
Xylem parenchyma	Warty surface	Smooth
Raphide	In cortex and pith	In cortex only
Saponin	In cortex only	In both hypodermis and cortex
Schlerides	Rarely present in conjugative cells	Profuse in conjugative cells

Table 5: Fluorescence analysis of tuber powder of both species

Chemicals for treatment	*C. borivilianum*			*C. tuberosum*		
	Visible light	Short wave UV(254nm)	Long wave UV(366nm)	Visible light	Short wave UV(254nm)	Long wave UV(366nm)
Pure dry powder	Creamy white	Greenish White	Whitish	Creamy brown	Greenish Brown	Whitish Brown
Powder in water	No change in colour	Light cobalt Green	Dark Violet	No change in colour	Light green	Light warm gray
P + Ab.Etoh	Antique White	Luminous Green	Light Violet	Light Brown	Whitish green	Violet
Methanol	No change in colour	Green	Dark Violet	No change in colour	Light green	Light Violet
P + n-buol	Antique White	Whitish Green	Crimson Red	Deep Brown	Whitish green	Violet
P + n-Hexane	No Change	Whitish Green	Pale violet red	Brown	Light green	Light violet
P + 1N Naoh	Off white	Grass Green	Light Violet	Light Brown	Green	Blackish
P + 50% Naoh	Deep Yellow	Pale Grass Green	Brownish Violet	Narcissus/ Yellow brown	Cobalt green	Amethyst Violet
P + 1N KOH	Brown Yellow	Green	Dark slate gray	Canary Yellow	Cobalt green	Light warm gray
P + NH4 Sol.	Light Cream	Light Cobalt Green	Crimson Red	Narcissus/ Yellow brown	Yellow Green	Light warm gray
P + GA	Light Cream	Light Cobalt Green	Moulin Rouge	Golden Yellow	Yellow Green	Violet
P + 1N Hcl	Golden Yellow	Green	Olive Green	Light Cream	Light Green	Warm gray
P + 50% Hcl	Light Brown	Green	Warm Gray	Narcissus/ Yellow brown	Yellow Green	Amethyst Violet
P + Conc. Hcl	Light Brown	Olive Green	Amethyst Violet	Brown	Yellow Green	Amethyst violet
P + 50% H2SO4	Light Brown	Yellow Green	Violet	Light Brown	Yellow Green	Prussian Blue
P + Conc. H2SO4	Golden Brown	Light Green	Black Violet	Deep Brown	Deep Green	Prussian Blue
P + 50% HNO3	Deep Yellow	Light Green	Brown Violet	Golden Brown	Green	Amethyst Violet
P + Conc. HNO3	Whitish Yellow	Light Green	Warm Gray	Golden Yellow	Golden Green	Amethyst Violet
P +Acetone	No change	Grass Green	Brownish Violet	No change	Cobalt green	Violet
P + 5% Iodine	Terra Cotta	Green	Brown	Light Yellow	Light Green	Light Warm Gray
P + 5% Fecl3	Brown Yellow	Green	Blue Violet	Golden Brown	Deep Green	Amethyst Violet

Table 6: Presence of active components in two species

Secondary metabolites	*C. borivilianum*	*C. tuberosum*
Alkaloids	+	+
Flavonoids	-	-
Steroids	+	+
Glycosides	+	+
Phenols	-	-
Saponins	+	+
Triterpenoids	+	+
Starch		
Anthraquinone glycosides	-	-

DISCUSSION

Both of the species were noted to be strikingly similar in look, however, flower was realized to be the key part for their morphological discrimination and ready recognition in the field (fig 1 & 2). Leaves and tubers showed some differences in gross appearance as well as in other details. Leaf margin of *C. borivilianum* is straight, whereas, in *C. tuberosum* it is wavy and the tubers of *C. tuberosum* were recorded to be longer than *C. borivilianum* (fig 3). Distinctiveness of two species was found to be apparent in respect of number of xylem strand, presence of saponin, raphides and sclereides in tuber anatomy (table 3 & 4 fig 4, 7 & 8). Panda [15] depicted six vascular bundles in *C. borivilianum*, while it was noted to be 9 – 10 in this study; in contrast to 11 – 15 in *C. tuberosum.* The latter species was noted to differ from *C. borivilianum* in having more amount of schleride in their conjugative cells. Bundle of raphides were rarely noticed in the cells of pith, only of *C. borivilianum* tuber, in longitudinal section. Tuber powders of two species were detected to have difference in colour, odour, taste and texture (table 1). Microscopic studies revealed the presence of both of reticulate and scalariform trachea in C. borivilianum, whereas, it was only scalariform in the other species; the fact also gets support from earlier work [15]Albeit the chemical similarity of saponin it was marked in hypodermis and cortex cells of *C. tuberosum* and only in cortex cells in *C. borivilianum* (fig 4 & 5).

On treating the tuber powder with different chemicals a variety of colors developed in presence of visible and ultra violate light (254nm & 366nm) helped two species be discriminated. Relevance of such findings also gets support from earlier works on *C. tuberosum* [16]. FTIR and HPTLC analyses even revealing the presence of same type of saponin in both of the species showed more amount of the chemical in *C. borivilianum* than *C. tuberosum* (fig 10 & 11). Two species were noted to have related functional groups, pertinence of such work gets support from like findings of earlier workers [17]. Both of the species were also found to have same type of secondary metabolites (table 6).

CONCLUSION

While consanguinity of two congeneric species of *Chlorophytum* has been revealed in the present study, right from the gross look of the species, their morphology, anatomy and even up to the details of chemicals present, some finer differences recorded, at different levels, provide the nuances with which two medicinally important species can be delimited from each other. Multifaceted characterization of two species done here will also help circumscribe two species more appropriately for the purpose of their use in drug preparation and so also in detecting adulterants, if any, mixed with them.

ACKNOWLEDGEMENT

We are thankful to Rajiv Gandhi National Fellowship Scheme (UGC) file no: 14-2(SC)/2008(SA -III) for providing financial support to carry out this research.

REFERENCES

1. Geetha KA, Maiti S: Characterization, genetic improvement and cultivation of *Chlorophytum borivilianum* – an important medicinal plant of India. Plant Genetic Resources 2005; 3: 264-272.
2. Mandal GD, Nandi AK: A new report on the occurrence of *Chlorophytum tuberosum* (Roxb.)Baker in the Districts of Bankura and Paschim Medinipur of West Bengal. Journal of Non-Timber Forest Products. 2012; 19(2): 103-106.
3. Mandal GD, Nandi AK: Morphological and anatomical circumscription for the identification of two source plants of aphrodisiac medicine *Chlorophytum borivilianum* Santapau & Fernandes and *Chlorophytum tuberosum* (Roxb.)*Baker*. Int. J. Med. Arom. Plants. 2012; 2(3): 406-410.
4. Banerjee G, Mukkherjee A. In Mahashwari, J.K. and Jain, A. P. (Eds.) Recent Researches in Plant Anatomy and Morphology. Pharmacognostic studies on *Portulaca oleracea* L. leaf. Scientific Publishers (India), Jodhpur: 2001.
5. Gupta HC, Kumar S and Rastogi DP. In Maheshwari, J.K. and Jain, A. P. (Eds.) Recent Researches in Plant Anatomy and Morphology. Comparative morpho-histological studies on two *Jalapas*. Scientific Publishers (India), Jodhpur; 2001.
6. Anonymous. Indian Pharmacopeia. Vol. I & II. Government of India, Ministry of Health and Family Welfare. The Controller of Publications, Civil Lines, Delhi, 1996.
7. Remya KV, Shirmila JG and Radhamany PM: Pharmacognostic and phytochemical investigation of *Hybanthus enneaspermus* linn. Int. J. of Pharmacy and Pharma. Sc. 2012;4(4): 77-80.
8. David Prain: Bengal Plants. N W & CO publication. 1903; 1: 1-675.
9. Iyengar MA, Nayak SGK. Anatomy of Crude Drugs. 7th ed. Manipur: M P Press; 1988. P1-77.
10. Kokoski CJ, Kokoski RJ, Sharma M: Fluorescence of powdered vegetable drugs and ultraviolet radiation. J. Am. Pharm. Ass. 1958; 47:715-717.
11. Harborne J.B: Phytochemical methods, A guide to Modern Techniques of plant analysis, 3rd edition, Springer (India) Pvt. Ltd., New Delhi, 1998:5-12.
12. Laha PK. Practical Phermacognosy. 1st ed. Lina Publication; 1981. p 1-200.
13. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Elsevier; 2002; p1-585.
14. Anonymous. The Ayurvedic Pharmacopoeia of India. Ministry of Health and Family Welfare, Govt. of India. 1989; 1(1): 138-145.
15. Panda SK, Das D and Tripathy NK: Pharmacognostical Studies on Root Tubers of *Chlorophytum borivilianum* Santapau & Fernandes. Int. J. of Pharma. and Phyto. Res. 2010; 2(4): 13-17.
16. Patil VN, Deokule SS: Pharmacognostic study of *Chlorophytum tuberosum* Baker. Int. J. Aur. Res. 2010; 1(4): 237-242.
17. Sharma V, Paliwal R: Isolation and characterization of saponins from *Moringa oleifera* (Moringaeceae) pods. Int. J. of Pharmacy and Pharma. Sc.; 2013; 5(1): 179-183.