

COMPARATIVE PHARMACOGNOSTICAL EVALUATION OF THREE SOURCE DRUGS OF TRIVRUT

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Abstract

Trivrut, considered as best among the laxative drug. Shyama, Aruna, Shukla etc. are the varieties delineated in different classical texts of Ayurveda. But clear morphological characters of the varieties are not described. Ayurvedic Pharmacopoeia of India (API) recommends *Operculina turpethum* (Linn.) Silva Manso (Convolvulaceae) as the source drug for Trivrut. In the herbal market, roots and stems of *Marsdenia tenacissima* Wight & Arn. (Asclepiadaceae), is being sold under the name of Shweta trivrut. Roots of *Operculina petaloidea* Choisy (Convolvulaceae) is also considered as Shyama trivrut. Present study was carryout to establish certain botanical standards for identification and standardization of three source drugs of Trivrut. Roots of the three samples were collected from the natural habitat, Odisha. Detailed morphological, microscopic and histochemical study was carried out following standard procedure. Differences in the morphological characters in individual sample were observed. Diagnostic character of the transverse section of *Operculina turpethum* shows the presence of Inter xylary phloem. Thin cork cells with abundant rosette and cluster crystals were observed in *Operculina petaloidea* whereas, *Marsdenia tenacissima* shows thick cork cell with abundant stone cells and starch grains. Test for Lignin, calcium oxalate crystal, starch grain and tannin showed positive result in all the three samples.

Key words: Trivrut; *Operculina turpethum*; *Operculina petaloidea*; *Marsdenia tenacissima*; Pharmacognosy.

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INTRODUCTION

Trivrut, known as Nishotha in Indian herbal market, is one of the important and frequently used herbal drug, in traditional system of medicine and has been considered as best among the laxative drugs (sukhavirechana) in Ayurveda.^[1] There are nearly 769 herbal and herbomineral formulations, used in Ayurvedic medicine, which contain Trivrut as an ingredient.^[2] Shyamatrivrut considered as one of its variety reported in near about 190 different formulations.^[3] Shyama (black), Aruna (redish), Shukla (white) etc. are the varieties delineated in different classical texts of Ayurveda. But clear morphological characters of the varieties are not described in the classical texts of Ayurveda. Thus, difference in the opinion for the variety of Trivrut has created lot of confusion in the authentic botanical source for the varieties of Trivrut. Further, the writers on Unani materia medica contribute confusion by mentioning Vidhara as synonyms with Nishottara.^[4]

Modern monographs on medicinal plants also equated white and black variety of turpeth. The description provided to the white variety is applicable to *Operculina turpethum* (Linn.) Silva Manso (Convolvulaceae). Ayurvedic Pharmacopoeia of India (API) also recommends the same drug as the source drug for Trivrut.^[5] Roots and stems of *Marsdenia tenacissima* Wight & Arn. (Asclepiadaceae), a source drug of Murva, as per API, is being sold under the name of Shweta Trivrut in the herbal market^[6]. Roots of *Operculina petaloidea* Choisy (Convolvulaceae) is also considered as Shyama Trivrut^[7], which is again considered as one of the source for Vidhara.^[4] Recent literature review shows that *O. turpethum*, *O. petaloidea* and *M. tenacissima* have been studied for pharmacognostical studies of their official part i.e. root. Comparative pharmacognostical study of the source drugs of Shukla Trivrut (white) viz. *O. turpethum* and *M. tenacissima* have also been carried out,^[8] but till date

comparative study on the source drug of Trivrut and Shyama Trivrut is not carried out. Hence, the present study was undertaken to establish certain botanical standards for identification and standardization of three source drug of Trivrut. The present research article will be helpful in distinguishing the roots characters and selecting the correct botanical source.

MATERIAL AND METHODS

Collection of drugs

Operculina turpethum, *Operculina petaloidea* and *Marsdenia tenacissima* growing naturally at the footrest of Nrusingnath temple, adjacent to Gandhamardana Hill Ranges, Balangir district of Odisha were identified by local taxonomist and their respective botanical name were confirmed by studying the morphological characters of various parts and comparing them with various characters described in floras and books.^[9] After identification and confirmation of the species, these three plant samples were collected, cleaned to remove adherent soil and dirt. The herbarium of respective drugs were prepared and stored in the pharmacognosy laboratory for further documentation (*O. turpethum* 6101phm, *O. petaloidea* phm 6069, *M. tenacissima* phm 6102). Collected roots were separated and washed with running fresh water and some of the pieces of each root were stored in AAF (70% Ethyl alcohol: Glacial acetic acid: Formalin) solution in the ratio of 90:5:5 for further study.^[10] Remaining roots were chopped; shade dried and pulverized using an electric blender. The powder was sieved through mesh size 60 and stored in an airtight food grade plastic container for further use.

Pharmacognostical study

Morphological characters including its shape, surface, colour etc. were studied by observing the root.

Morphology of root



Figure 1



Figure 2

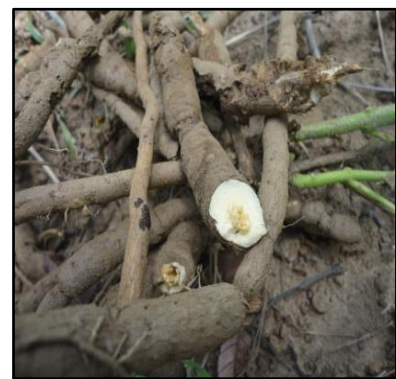


Figure 3

Transverse section of the root showing cork cortex and xylem

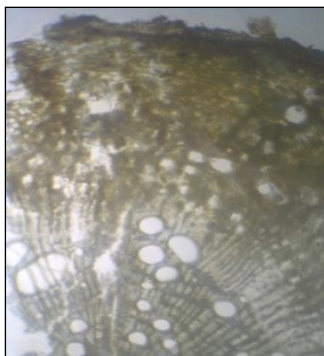


Figure 4

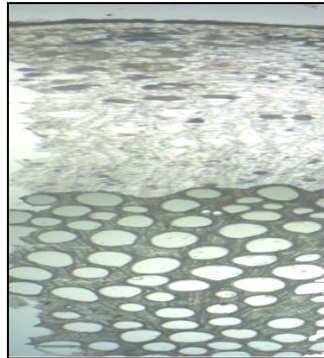


Figure 5

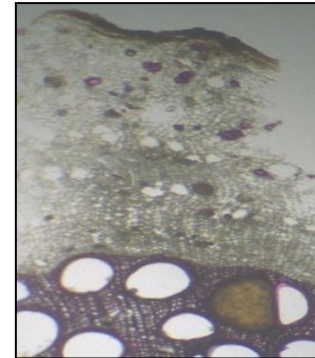


Figure 6

Interxylary phloem

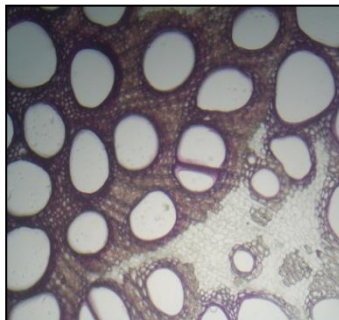


Figure 7



Figure 8

Intervascular pitting



Figure 9



Figure 10

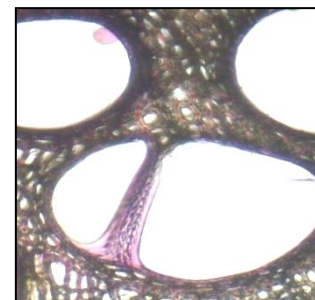


Figure 11

Crystals

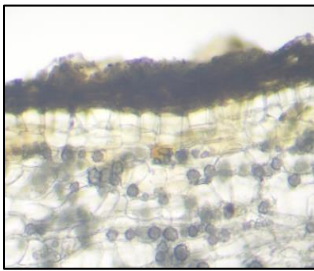


Figure 12

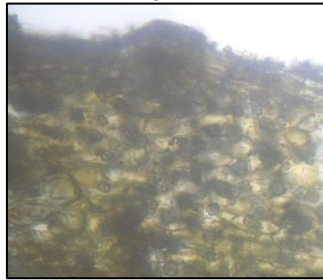


Figure 13

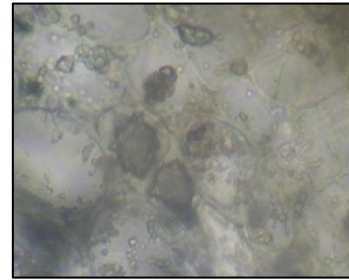


Figure 14

Lactiferous cells

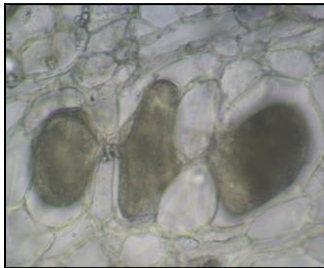


Figure 15

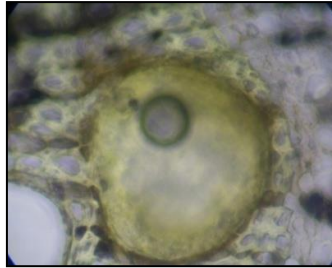


Figure 16

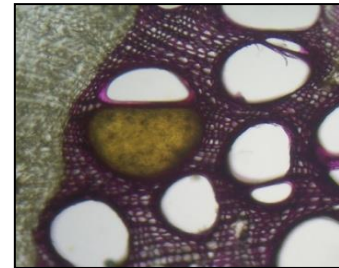


Figure 17

Powder



Figure 18



Figure 19



Figure 20

Rosette crystals/cluster Crystals



Figure 21



Figure 22



Figure 23

Stone cell



Figure 24



Figure 25



Figure 26

Starch grain



Figure 27



Figure 28



Figure 29

- Figure 1** Roots of *Operculina turpethum*
Figure 2 Roots of *Operculina petaloidea*
Figure 3 Roots of *Marsdenia tenacissima*
Figure 4,5,6 Transverse section of the root showing cork cortex and xylem
Figure 7 Interxylary phloem present in transverse section of the *Operculina turpethum*
Figure 8 Interxylary phloem present in transverse section of the *Operculina petaloidea*
Figure 9 More Intervascular pitting in T S of *Operculina turpethum*
Figure 10 Medium Intervascular pitting in T S of *Operculina petaloidea*
Figure 11 Less Intervascular pitting in T S of *Marsdenia tenacissima*
Figure 12 Rosette crystals present in *Operculina turpethum*
Figure 13 Abundant crystals present in *Operculina petaloidea*
Figure 14 Rosette crystal present in *Marsdenia tenacissima* but less in number as compared to *O turpethum*
Figure 15 Medium sized lactiferous cells present in *Operculina turpethum*
Figure 16 Latex content present in lactiferous cells of *Operculina petaloidea*
Figure 17 Bigger size lactiferous cells present in *Marsdenia tenacissima*
Figure 18 Dark brown colour powder of *Operculina turpethum*
Figure 19 Light brown colour powder of *Operculina petaloidea*
Figure 20 Whitish colour powder of *Marsdenia tenacissima*
Figure 21 Rosette crystals present in *Operculina turpethum*
Figure 22 Abundant Rosette crystals present in *Operculina petaloidea*
Figure 23 Less amount of Rosette crystals present in *Marsdenia tenacissima*
Figure 24 Lignified pitted stone cells with narrow lumen present in *Operculina turpethum*
Figure 25 Lignified pitted stone cells with wide lumen present in *Operculina petaloidea*
Figure 26 Lignified stone cell with yellow content present in *Marsdenia tenacissima*
Figure 27 Simple and compound (2-3) starch grains with central cleft hilum present in *Operculina turpethum*
Figure 28 Simple and compound (3-5) starch grains with central cleft hilum present in *Operculina petaloidea*
Figure 29 Simple and compound (2-3) starch grains present in *Marsdenia tenacissima*

Table 1: Showing the morphological characters of the *O. turpethum*, *O. petaloidea*, *M. tenacissima* root

Morphological characters	<i>Operculina turpethum</i>	<i>Operculina petaloidea</i>	<i>Marsdenia tenacissima</i>
Shape	Cylindrical	Irregular	Cylindrical
Surface	Rough, unbranched and bears thin rootlets	Rough exterior, scars of rootlets present	Longitudinal ridges and furrows present
Colour	Dark brown	Light brown	Dark brown
Bark	Easily peeled off, thin bark	Easily peeled off, thin bark	Slightly difficult to peeled off, thick bark
Latex	Present	Present at cut region	Absent
Central portion	Whitish, less fibrous	Light yellow in colour, woody	Light yellow in colour. More fibrous

Table 2: Microscopic characters of transverse section of the *O turpethum*, *O petaloidea*, *M tenacissima* roots

Pharmacognostical characters	<i>O. turpethum</i>	<i>O. petaloidea</i>	<i>M. tenacissima</i>
Cork	Thin cork, consisting of 3-5 rows of brown Cells. Phelloderm 4-5 layers of tangentially elongated cells	Compactly arranged lignified cork, composed of 8-10 layered tangentially arranged barrel shaped and elongated cells	Composed of 15-25 layers of thin-walled, tangentially elongated, rectangular cells, some filled with reddish-brown contents
Cortex	Cortex 4-6 layered, composed of tangential elongated, thin-walled cells. Secretory cavities surrounded by subsidiary cells.	Parenchymatous cell filled with simple and compound starch grains, clustered crystals and oil globules, Laticiferous cell	Composed of an outer region of broken ring of stone cells of varying thickness, followed by wide zone of oval to polygonal parenchymatous cells;
Sec. Phloem	Thick, consist sieve elements and phloem parenchyma	Sieve elements, fibres, starch grains, cluster crystal and uni to biseriate medullary rays	Mostly parenchyma with small patches of sieve elements and small strands of stone cells
Xylem	3-5 radiating arms with Intraxylary phloem	3-5 group of xylem vessel, xylem fibres along with Intraxylary phloem, latex cell	Wedge-shaped structure, consisting of parenchymatous tissue, lignified tissue, vessels, tracheids, fibres, and xylem parenchyma.
Sec xylem	With tracheids, xylem parenchyma and its fibres	Large tracheids, xylem parenchyma, parenchyma and wood fibre	vessels, tracheids and uni to biseriate medullary rays
Stone cell and pericyclic fibre	Present	Present	Present, yellow in colour, pitted.
Lysogenous cavity/	Secretory cavities with subsidiary cells singly or circle.	Present with intraxylary phloem	Absent
Laticiferous tubes	Appeared in the cortical zone	Appeared in the cortical zone	Present with intraxylary phloem
Vascular bundle	Arranged in circle, uni-biseriate medullary rays	Arranged in circle, uni-biseriate medullary rays	Circularly arranged with uni to multiseriate medullary rays
Sec.Cortex	Secretory cavities surrounded by subsidiary cells and resin canals	Secretory cavities surrounded by subsidiary cells.	Stone cells, oval to polygonal parenchymatous cells
Intervascular pitting	Present	Present	Absent
Interxylary phloem	Present, often formed	Present	Absent
Crystals	Rosette and prismatic crystal	Rosette, cluster and prismatic crystal	Rosette, cluster and prismatic crystal
Starch grains	Simple and compound with hilum	Simple and compound with hilum	Simple and compound starch grain with hilum
Tracheids	Blunted with lumen	Blunted with lumen	Narrower with tapering end
Xylem fibre	Narrow, wavy, Boredr pitted	Narrow, wavy, Border pitted	Narrow, wavy, pitted

For detailed microscopical observation, thin free hand transverse sections were taken and cleared with chloral hydrate and observed as such for the presence of any crystals, then were stained with phloroglucinol and concentrated hydrochloric acid to notice the lignified elements like fibres, vessels etc.

following standard procedure.^{[11][12]} Histochemical tests were carried out by taking thick sections following the standard procedure methods.^[13] The sections were stained with various reagents like phloroglucinol followed by HCL for lignified elements, iodine for starch grains etc. Photographs of the sections were taken with the help of canon Ixus 130

camera attached to Carl-zeiss Trinocular Microscope. Powder of individual sample was used for powder microscopy following standard procedure.

RESULTS

Macroscopy

Roots of *O. turpethum*, *O. petaloidea*, *M. tenacissima* were observed for morphological characters like shape, size, colour etc. Cylindrical shape was observed in *O. turpethum* and *M. tenacissima* root, whereas irregular root with scars of rootlets was observed in *O. petaloidea*. (Table 1) (Figure 1, 2 and 3)

Microscopy

Transverse sections of fresh roots of each drug was taken and observed under microscope for identical, similar and dissimilar characters. Thin cork was observed in *O. turpethum* and *O. petaloidea*, whereas thick cork was observed in *M. tenacissima*. (Figure 4, 5 and 6) Interxylary phloem was absent in *M. tenacissima*. Intervascular pitting was more in *O. turpethum* whereas, abundant rosette crystals were present in *O. petaloidea* as compared to *O. turpethum* and *M. tenacissima*. (Figure 7 to 14) (Table 2)

Organoleptic characters

Characteristic differences in the organoleptic characters like colour, taste, touch were observed in individual powder sample (Figures 18, 19 & 20). Bitter taste was present in the powder of *M. tenacissima*, whereas powder of *O. turpethum* and *O. petaloidea* was nasal irritant in nature. (Table 3)

Powder microscopy - *O. turpethum*

Simple fiber, rosette crystals, tracheids, tannin, stone cells, cellulosic fibres with pointed tips, vessels with simple pits, simple

and compound starch grains elliptical to spherical with central cleft.

O. petaloidea

Simple fiber, rosette crystals, scleroids, laticiferous cell, parenchyma, tracheids and tannin.

M. tenacissima

Yellow coloured stone cells, xylem, fibres, tracheids, vessel with pitted walls, fragments of cork, rosette and prismatic crystal of calcium oxalate, simple and compound starch grains. (Figure 21 to 29)

Histochemical study

Test for Lignin, calcium oxalate crystal, starch grain and tannin showed positive result in all the three samples. (Table 4)

DISCUSSION

Morphological and microscopical study of the three source plants of Trivrut show some similar as well as some distinct characters. *O. turpethum* and *O. petaloidea* belonging to same family shows some similar characters like habit, flower aestivation etc. and differ in the characters like pubescent outer sepals and glabrous seeds are the key characters of *O. turpethum* whereas, glabrous outer sepals, minutely velvety seeds with subvillous margin are the key characters of *O. petaloidea*. *M. tenacissima* belonging to Asclepiadaceae family is sold in the market though it is not having purgative action. Transverse section of the roots of these three species shows some similar and some dissimilar characters. Presence of starch grains with hilum, intervacular pitting, tannin content, laticiferous cells and calcium oxalate crystal are some of common characters present in all the three samples. Transverse section of the roots of these three species shows some similar and some dissimilar characters.

Table 3: Showing comparative organoleptic characters of root powder

Organoleptic characters	<i>O. turpethum</i>	<i>O. petaloidea</i>	<i>M. tenacissima</i>
Colour	Dark brown	Light brown	Buff colour
Taste	Astringent, bitter with tingling sensation	Indistinct with tingling sensation	Bitter
Touch	Smooth	Smooth	Very fine
Odour	Indistinct	Nasal irritant	Characteristic

Table 4: Showing the result of histochemical tests

Reagents	Test for	<i>O. Turpethum</i>	<i>O. petaloidea</i>	<i>M. tenacissima</i>
Phloroglucinol +Conc HCL	Lignin	+	+	+
Phloroglucinol +Conc HCL	Calcium oxalate crystal	+	+	+
Iodine	Starch	+	+	+
Ferric chloride solution	Tannin	+	+	+

Presence of starch grains with hilum, intervascular pitting, tannin content, laticiferous cells and calcium oxalate crystal are some of common characters present in all the three samples. *Operculina petaloidea*, known as kali tihudi by the local people of Odisha is being used for its purgative effect. *O. petaloidea*, is considered as one of the source for Vidhara,^[4] but *Argeria speciosa* syn. *Argeria nervosa* is now considered as the authentic source for Vruddhadaru.^[14] Therefore, *O. petaloidea* can be considered as a source drug for Shyama Trivrut after detail scientific evaluation for reported clinical claims.

CONCLUSION

Presence of Interxylary phloem in *Operculina turpethum*, Thin cork cells with abundant rosette and cluster crystals in *Operculina petaloidea*, thick cork cell with abundant stone cells and starch grains in *Marsdenia tenacissima* can be considered as the diagnostic characters of the individual sample.

REFERENCES

- Caraka. Caraka Samhita (Vidyotini Hindi Commentary), Vol. 1. Acharya Yadavji Trikamji, editor. 1st ed. Varanasi: Caukhambha Bharati Academy; 2011. Sutrasthana, 25/40, p.132.
- Kolhe Rasika, Acharya RN. Trivrut and its importances in the classical texts of Ayurveda – A comprehensive review. Research and Reviews: Journal of Ayurvedic Science, Yoga and Naturopath.,2014; 1(2): 1-31
- Kolhe Rasika, Acharya R N. Shyamatrivrut, less known but frequently used drug of Ayurveda: A review. Global J Res Med Plants & indigen Med. 2014;2(11):772–784.
- Raghunathana, Roma Mitra. Pharmacognosy of indigenous drugs, Vol II. 2nd ed. New Delhi: CCRAS; 2005. p.1085.
- Anonymous. Ayurvedic Pharmacopoeia of India, Vol. III. 1st ed. New Delhi: Government of India, 2001.p.215.
- Bapalal Vaidya. Some controversial drugs in Indian Medicine. 2nd ed. Varanasi: Chaukhambha Orientalia; 2005.p. 128.
- Khare CP. Indian medicinal plants, 1st ed. Delhi: Springier; 2007.p.334.
- Raghunathana, Roma Mitra. Pharmacognosy of indigenous drugs, Vol. II. 2nd ed. New Delhi: CCRAS; 2005. p.960-975.
- Saxena HO. Flora of Orissa. 1st ed. Bhuvneshwar: Regional research laboratory; 1995. p.1198.
- Johnson Alexander Donald. Plant Micro technique. 1st ed. New York: McGrow Hill Book Company; 1940.p.105.
- Trease GE, Evans WC. Pharmacognosy. 16th ed. Saunders: Elsevier; 2009.p.309–310.
- Wallis TE. Text book of Pharmacognosy. 5th ed. New Delhi: CBS Publishers; 1985. p.572–578.
- Krushnamurthy. Methods in the plant histochemistry. 1st ed. Madras: Vishwanadhan Pvt Limited; 1988. p.1–77.
- Nadkarni KM. Indian Materia Medica, Vol. I. 1st ed. Bombay: Bombay Prakashana Private limited; 1996.p.136.