ABSTRACT

Objective: This study was undertaken to carry out a comparative pharmacognostical evaluation of three botanical source plants used in the name of classical Ayurvedic drug Jivanti.

Methods: Leaves of three source plants of jivanti belonging to Asclepiadaceae family i.e. Leptadenia reticulata (Retz.), Holostemma ada-kodien Schult. and Wattakaka volubilis (Linn. f.) Stapf were evaluated for morphological and microscopical characters including quantitative microscopy, surface study, powder microscopy and histochemical studies.

Results: Morphologically all the three species showed some similar characters like simple, opposite leaves with reticulate venation. The shape of the leaves were ovate to oblong in L reticulata and H ada-kodien whereas W volubilis was having broadly ovate or suborbicular leaves. Multicellular glandular warty trichomes, rosette crystals of calcium oxalate, lactiferous cells were observed in all the three species whereas prismatic crystals were also present in W volubilis. Palisade ratio and stomatal index were higher in H ada-kodien followed by L reticulata. Characteristic differences in the organoleptic characters like colour, taste, touch were observed in individual powder samples. Test for lignin, calcium oxalate crystal, starch grain and tannin showed a positive result in all the three species.

Conclusion: The present study reports specific pharmacognostical characters for the identification and differentiation of each botanical source plant. The observed results can also serve as a reference for any further investigations.

Keywords: Holostemma ada-kodien Schult., Jivanti, Leptadenia reticulata (Retz.), Pharmacognosy, Wattakaka volubilis (Linn. f.) Stapf.
and Hydrochloric acid to notice the lignified elements like fibers, vessels etc [8, 9]. Photographs of the section were taken with the help of Canon digital camera attached to Zeiss microscope.

**Quantitative microscopy**

Quantitative microscopy was carried out to determine epidermal cell number, stomatal number, stomatal index and size of the stomata [10].

**Powder microscopy**

Dried leaf powder of all the three species was studied following standard procedures [11]. The microphotographs were taken by using Carl zeiss trinocular microscope.

**Histochemical test**

To confirm the presence and absence of the chemical constituents the material were subjected to various tests. The histo-chemical tests were carried out according to the standard guidelines of practical pharmacognosy [12].

**RESULTS AND DISCUSSION**

**Morphology**

Morphologically all the three plants are distinct in their appearance and can be easily identified. Leaves of all the three species belonging to Asclepiadaceae family, are simple and opposite. Stipules are very small or absent in L reticulata whereas H ada-kodien and W volubilis were ex-stipulated. Comparatively, the petiole is larger in W volubilis and H ada-kodien. The shape of the leaves was ovate to oblong in L reticulata and H ada-kodien whereas W volubilis is having broadly ovate or suborbicular leaves. Leaves of all the three species belonging to Asclepiadaceae family showed reticulate venation with 4-6 pairs of nerves. The texture of leaves was hirtellous in L reticulata whereas in H ada-kodien and W volubilis, leaves were glabrous above and pubescent beneath. The shape of the apex was acuminate in W volubilis, cuspidate in both L reticulata and H ada-kodien. Leaves of L reticulata were having obtuse or sub cordate base whereas the shape of the base was deeply cordate and rounded in H ada-kodien and W volubilis respectively (table 1).

**Table 1: Comparative morphology of leaves of three source plants of jivanti**

| S. No | Parameter          | Results                  | L. reticulata | H. ada-kodien | W. volubilis |
|-------|--------------------|--------------------------|---------------|---------------|--------------|
| 1     | Type               | Simple                   | Simple        | Simple        | Simple       |
| 2     | Phyloaxy           | Opposite                 | Opposite      | Opposite      | Opposite     |
| 3     | Stipules           | Very small or absent     | Exstipulate   | Exstipulate   | Exstipulate  |
| 4     | Petiole            | 1.1-2.1 cm               | 2.8-4.5 cm    | 3.2-5.8 cm    | Broadly ovate or suborbicular, 6.5-14/4.5-11 cm |
| 5     | Shape and size     | Ovate to oblong, 5-4.7 cm| Oblong-ovate, 8.2-11×3-5.6 cm, upper leaves somewhat triangular | Broadly ovate or suborbicular, 6.5-14/4.5-11 cm |
| 6     | Venation           | 6 pairs of nerves with reticulate venation | 4-Spairs of nerves with reticulate venation | Reticulate venation |
| 7     | Texture            | Hirtellous above         | Glabrous above, thinly pubescent beneath | Glabrous above, less softly pubescent beneath |
| 8     | Apex               | Cuspidate                | Cuspidate     | Acuminate     |              |
| 9     | Base               | Obtuse or subcordate     | Deeply cordate, 5-7 glands at the base of midrib | Rounded, few small glands just above the petiole |

**Table 2: Comparative microscopical characters of three source plants of jivanti**

| Parameter | Results                          | L. reticulata | H. ada-kodien | W. volubilis |
|-----------|----------------------------------|---------------|---------------|--------------|
| Petiole    |                                  |               |               |              |
| Epidermis  | Circular                         | Deeply concave in upper side | Circular      |              |
| Epidermal cells | Single layered                   | Single layered | Single layered |              |
| Cuticle    | Thin                             | Thin walled and small | Thin walled and small | Thin          |
| Trichomes  | Multilayered glandular           | Multilayered | Multilayered | Multilayered |
| Hypodermis | 3-4 layers of circular to oval collenchyma cells with angular thickenings | 2-3 layers of collenchyma cells | 3-4 layers of collenchyma cells |
| Cortex     | Thin-walled circular to oval parenchymatous cells with distinct intercellular spaces | 5-6 layers of parenchymatous cells | Thin-walled circular to oval parenchymatous cells with large intercellular spaces |              |
| Crystals   | Several prismatic crystals are present | Rosette and prismatic crystals of calcium oxalate | Several prismatic, rosette crystals are present |              |
| Vascular bundle | Crescentic bicollateral vascular bundle, xylem is located in the centre followed by phloem on both sides | Arranged in crescentic shape in the middle, separated by wide areas of ground tissue. | Bicollateral vascular bundle, protoxylem facing towards center and metaxylem towards the epidermis. |              |
| Midrib     |                                  |               |               |              |
| Upper epidermis | Barrell-shaped | Oval to rectangular | Barrell-shaped |              |
| Epidermal cell | Multilayered glandular trichomes | Multilayered | Multilayered | Multilayered |
| Trichomes  | Thick                           | Moderate      | Single layered, elongated barrel shaped palisade parenchyma cells with numerous chloroplasts | Thick         |
| Cuticle    | 1-2 layers of compactly arranged palisade parenchyma with oil globules and rich in chloroplast | Prismatic crystals | 1-2 layers of palisade parenchyma cells with chlorophyll pigments and oil globules |
| Hypodermis | Rosette crystals of calcium oxalate | Open and bicollateral vascular bundle | Open and bicollateral vascular bundle |              |
| Crystals   |                               |               |               |              |
| Vascular bundle |                              |               |               |              |
Microscopy

Among the three source plants of jivanti, studied for their microscopic characters, the common characters of Asclepiadaceae family and some individual microscopic characters were observed. All these microscopical characters can be used for identification of the species and to differentiate each other. T S of H ada-kodien was deeply concave in upper side whereas in W volubilis and L reticulata T S was circular in shape. Epidermis was single layered in all the three species. Cuticle layers are thick in H ada-kodien, L reticulata and W volubilis composed of thin cuticle. Cuticular characters of Asclepiadaceae family like crescenteric bicollateral vascular bundles, multicellular glandular warty trichomes, prismatic and rosette crystals of calcium oxalate were seen in all the three species.

Transverse section of midrib of L reticulata, H ada-kodien and W volubilis was studied and compared for their identical and differential characters. T S of mid rib was strongly convex in L reticulata and W volubilis, broadly semicircular in H ada-kodien. Epidermis was single layered and covered with cuticle in all the three species. Epidermal cells were barrel-shaped in L reticulata and W volubilis, oval to rectangular shaped in H ada-kodien. Multicellular glandular warty trichomes, rosette crystals of calcium oxide, laticiferous cells were observed in all the three species whereas prismatic crystals were also present in W volubilis. All the three species showed centrally located, open, bicollateral vascular bundle.

Surface study

Surface study plays an important role in drug identification. The importance of epidermal characters, in general, is widely recognized in taxonomic considerations and in many cases, these are successfully used in the identification of taxa at genus as well as species levels [13]. Similarly, studies in stomata have a great taxonomic as well as pharmacognostic value in the proper identification of medicinal plants [11]. In the present study, stomata were rarely distributed in the upper epidermis in W volubilis whereas stomata were absent in L reticulata and H ada-kodien. Some of the trichomes and cicatrix were also observed in W volubilis. The lower epidermis composed of paracytic stomata, trichomes and cicatrix in L reticulata and H ada-kodien, paracytic and anisocytic stomata and in W volubilis.

Quantitative microscopy

All the three source plants composed of a paracytic type of stomata. Size of laticiferous cells, warty trichomes and epidermal cells were larger in H ada-kodien compared to other species. Size of the palisade cells was almost similar in L reticulata and W volubilis whereas it was much smaller in H ada-kodien. Cuticle layer was thicker in L reticulata. Length and the surface measurement of xylem fibres were more in L reticulata. Palisade ratio and stomatal index were higher in H ada-kodien followed by L reticulata (table 3).

Powder microscopy

Characteristic differences in the organoleptic characters like colour, taste, touch were observed in individual powder samples. Different organoleptic characters observed during the study are presented in table 4.

Histochemical study

Test for lignin, calcium oxalate crystal, starch grain and tannin showed a positive result in all the three samples. (Table 5).

Table 3: Comparative quantitative microscopy of leaves of three source drugs of jivanti

| S. No. | Parameter                          | Results          |
|-------|-----------------------------------|------------------|
| 1.    | Type of stomata                   | Paracytic        |
| 2.    | Size of stomata (Length X width)  | 0.60X0.30 μm     |
| 3.    | Laticiferous cavity (surface)     | 336.63 μm²       |
| 4.    | Xylem measurement from proto to metaxytem | 119.08 μm²     |
| 5.    | Xylem surface measurement         | 846.25 μm²       |
| 6.    | Rosette crystals                  | 329.31 μm²       |
| 7.    | Warty trichome                    | 3735.54 μm²      |
| 8.    | Palisade cell measurement         | 1040.41 μm       |
| 9.    | Epidermal cell measurement        | 217.11 μm²       |
| 10.   | Cuticle layer measures            | 32.42 μm²        |
| 11.   | Stomatal index                    | 25               |
| 12.   | Palisade ratio                    | 3                |

Table 4: Organoleptic characters of the three source drug of jivanti

| Organoleptic characters | Leptadenia reticulata W and A | Holostemma ada-kodien schult. | Wattakaka volubilis L. f |
|-------------------------|-------------------------------|------------------------------|--------------------------|
| Colour                  | Light green                   | Dark green                   | Light green              |
| Taste                   | Slightly bitter               | Sweet and bitter             | Slightly sweet and bitter|
| Touch                   | Smooth                        | Smooth                       | Smooth                   |
| Odour                   | Characteristic                | Characteristic               | Characteristic           |

Diagnostic character like paracytic stomata, rosette crystals of calcium oxalate, and laticiferous cells were observed in all the three species. L reticulata and H ada-kodien showed multicellular warty trichomes whereas multicellular and glandular trichomes were observed in Wattakaka volubilis.

Table 5: Histochemical study of three botanical source drug of jivanti

| Reagents            | Test for          | Observation          | L. reticulata | H. ada-kodien | W. volubilis |
|---------------------|-------------------|----------------------|---------------|---------------|--------------|
| Phloroglucino+Conc HCL | Lignin            | Red colouration      | ++            | ++            | ++           |
| Phloroglucino+Conc HCL | Calcium oxalate crystal | Dissolved            | ++            | ++            | ++           |
| Iodine              | Starch            | Blue                 | ++            | ++            | ++           |
| Ferric chloride solution | Tannin         | Blue–black colouration | ++            | ++            | ++           |

*++* Present
Fig. 1: Morphological characters of three source plants of jivanti

Fig. 2: Measurement of leaves

Fig. 3: T S of petiole

Fig. 4: T S of mid rib
A. *L. reticulata*

B. *H. ada-kodein*

C. *W. volubilis*

**Fig. 5:** T S of petiole showing vascular bundle

A. *L. reticulata*

B. *H. ada-kodein*

C. *W. volubilis*

**Fig. 6:** T S of petiole showing epidermis and hypodermis

A. *L. reticulata*

B. *H. ada-kodein*

C. *W. volubilis*

**Fig. 7:** T S of petiole showing trichomes

A. *L. reticulata*

B. *H. ada-kodein*

C. *W. volubilis*

**Fig. 8:** T S of petiole showing xylem fibres
CONCLUSION
The observed macroscopical and microscopical characters are useful for the identification and differentiation of closely related species used in the name of jivanti. The results of comparative quantitative microscopy are reported for the first time. These observations are specific to the species and can be considered as the diagnostic characters of the individual sample.

AUTHORS CONTRIBUTIONS
Dr Raghavendra Naik conceptualized, designed, carried out the work and drafted the article. Dr Rabinarayan Acharya, conceptualized, designed, monitored the work and edited the article. Dr Harisha C R supervised the experimental study and edited the manuscript.

CONFLICT OF INTERESTS
The authors do not have any conflict of interest to declare.

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