PHARMACOGNOSTICAL AND PHYTOCHEMICAL INVESTIGATION OF PERISTROPHE BICALYCLULATA

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Received: 23 September, 1992 Accepted: 14 December, 1992

ABSTRACT: Morphological, microscopical and phytochemical studies of root and stem of the herb Peristrophe bicalyculata (Acanthaceae) were carried out. Presence of coumarins, alkaloids, potassium chloride (stem and root), saponins and a free sugar (root) have been here in this herb for the first time.

INTRODUCTION

Peristrophe bicalyculata (Acanthaceae), commonly known as Kakjangha in Sanskrit and Kaliadhedi in Gujarat, is an annual herb wildly growing throughout India. It is one of the traditional herb recommended in cases of tuberculosis. In Uttar Pradesh, the paste of the plant is used for sprain and bone fracture. The herb is said to possess number of other therapeutic properties also, such as expectorant, analgesic, anti-inflammatory, antipuretic, antibacterial, etc. Number of formulations containing either root, seed or entire herb, have been mentioned in Ayurvedic Formulary. Further, it is said to be a good substitute for Fumaria parviflora (Fumariaceae) which is one of the reputed drug employed in cases of fever and skin diseases.

Some controversy existed regarding the correct identity of Kakjangha and besides Peristrophe bicalyculata, the other plants described under this common name are: Leea hirata (Vitaceae), Leea macrophylla (Vitaceae) and Vitex penduncularis (Verbenaceae), Peristrophe bicalyculata has been now correctly established as the true Kakjangha.

The various constituents reported in the plant are: Volatile oil (herb), pentunidin – 3 – rhamnoglucoside (flower), sterols, fatty acids (stem and root), free amino acids and free sugars (stem). Except these constituents, none of the other chemical constituents have been reported so far in any part of this plant. The volatile oil of this herb, is reported to possess the antitubercular activity.

MATERIALS AND METHODS

Fresh entire mature herbs were uprooted from the vicinity of the College in the month of October to December 1992, when they were in full bloom. The authenticity of the herb was confirmed by studying the characters mentioned in the various floras and with the help of a taxonomist of the Botany Department, Gujarat University. Roots and stems were separated from the plant and dried in shade. 40 mesh powders were separately prepared from these and were used for the preliminary phytochemical screening and for the extraction and isolation of various compounds.
Microscopical Observations:

Free hand sections of stem and root were taken for histological studies and were stained with various reagents\textsuperscript{15} and macerates were prepared by Schultz Method\textsuperscript{16}.

Preliminary Phytochemical Screening:

The ethanolic extracts of the root and the stem were subjected separately to various chemical tests to detect the presence of alkaloids, coumarins, saponins, etc.

Extraction, Isolation and Testing Methods:

300g of stem and root powders were separately defatted with petroleum ether and the defatted powders were separately subjected to the process of isolation and extraction of the following compounds:

Alkaloids:

The usual procedure for the isolation of the alkaloids were adopted whereby the powders were separately basified with ammonium hydroxide and extracted with chloroform. The crude dried alkaloidal extracts obtained were resolved on TLC using silica gel G as a stationary phase, t-Butanol: Ethyl Acetate : Diethylamine (70:20:10), as a mobile phase and Dragendorff’s solution as a spray reagent.

Saponins, Coumarins, Inorganic Salts and Free Sugars:

The marc left after the removal of the alkaloids, was extracted with ethanol and the ethanolic extract was concentrated to about 50ml volume and cooled at $5^\circ C$ for 24 hours. White crystals settled were separated off and were subjected to physical and chemical testings. Free sugar obtained in the root was resolved on silica gel G impregnated with 0.1 M sodium bisulphate, using Ethyl Acetate: Acetic Acid: Methanol: Water (6:1.5:1.5:1) as solvent system and Thymol – Sulphuric Acid was used as a spray reagent.

Additional of acetone to the alcoholic extracts of stem and root, yielded buff coloured precipitates which were separated off and were purified by first dissolving in water and then extracting it with n-butanol. The n-butanol extracts was evaporated to dryness and was subjected to various chemical tests to detect the presence of coumarin glycosides and saponins. Coumarin glycosides were separated on silica gel G plate using Acetic Acid: Water (27.73) as a mobile phase and the plates were observed as such and then after spraying with ethanolic potassium hydroxide, under UV light. The saponins were separated on silica gel G plate using n-Butanol : Acetic Acid : Water (4 :1:5) as a mobile phase and Libermann – Burchard as a spray reagent. The mother liquors left out after the removal of buff coloured precipitate, were separately concentrated to about 50ml volume and the free coumarin aglycones present in this, were separated on silica gel G plate using Toulene: : Ether (1:1, saturated with 10% Acetic Acid) as solvent system and the plates were observed as such and then after spraying with ethanolic potassium hydroxide, under UV light.

RESULTS AND DISCUSSION

Morphology:

The axis of the stem is slender, ascending, six angled, winged and dichotomously branched. The portion slightly above the nodes get abruptly swollen and slightly bent towards the inner side of the axis. The convex side of this portion occasionally
show protruding small sharp spines arranged in semilunar fashion (Fig. 1A). Fracture is splintery with pithy centre which gets exposed in the form of powdery mass forming a hollow centre. Lenticles are few, prominent, placed both in furrowed and winged region of the old stem.

Leaves are green, ovate, opposite, simple, pubescent, short petiolate, acuminate, 5-9 cm long and 3-5 cm wide.

Roots are cylindrical, slender, some what tortuous, tapering and with numerous branching lateral rootlets. It measures 8 – 12 cm in length and 3 – 5 mm in diameter. Externally, it is pale brown, wrinkled longitudinally and bears numerous fibrous lateral roots (5 – 40 cm in length and 0.5 – 1.5 mm in diameter) or the scars left by them. The roots are hard with short uneven fracture, internally white, occasionally found to be infected with yellow coloured (2 – 3 cm long and 1 – 2 mm broad) worms. (Fig. 1B)

Histology:

T.S. of Stem :

The outline of the transverse section of the stem is six angled and showed a central pith and a continuous ring of xylem forming six vascular strands, each lying under the ridged portions of the section (Fig. 2A).

The epidermis is papillose and bears sessile glandular trichomes and diacytic stomata, similar to that of Vasaka leaf. Cortex is parenchymatous, except at the winged region, where lies the collenchymatous tissue. Endodermis, separating the stellar and the cortical tissue in distinct. Pholem forms a narrow band, encircling the continuous ring of xylem, which is composed of group of radially arranged isolated xylem vessels, fibres, tracheids and parenchyma. The central parenchymatous pith contains isolated prisms of calcium oxalate crystals (Fig. 2B).
Figure 2  
A - Diagramatic T.S. of stem (X15).
B - T.S. of stem passing through winged portion (X65).
C - Diagramatic T.S. of root (X24).
D - T.S. of root (X160).
T.B. of Root : (Fig. 2 C & D)

The central xylem region is very wide, occupying 75% of the total area of the section. Bark is narrow, consisting of well developed outermost lignified cork, 4 – 5 rows of collenchymatous broad celled tissue lying underneath this, a ring of endodermis, the cells of which occasionally getting sclerosed and a narrow phloem. Groups of nonlignified fibres (Characteristic of Acanthaceae family)17 were also found to be located at the peripheral regions of the phloem.

Preliminary Phytochemical Screening, Isolation and TLC separation of Various Compounds:

Preliminary Phytochemical screening of Peristrophe bicalyculata indicated the presence of alkaloids, coumarins and potassium chloride in root and stem parts of the plant. Triterpenoid Saponins and free sugars were also detected in root. Leaf is found to be devoid of all these compounds.

The various compounds isolated from root and stem, their percentage yield and TLC separation is mentioned in the Table.1.

Investigation of the antibacterial activity of the aqueous extract of the herb and its Kshara (an aqueous extract of the ash) is in progress and will be reported later on.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Ketan Amin of M/s. Ban Pharmaceuticals, Rajkot, for suggesting the present problem and providing the valuable suggestions. The authors are thankful to the Principal, L.M. College of Pharmacy, for providing the laboratory facilities for carrying out the present work.
### TABLE 1

Percentage Yield and TLC Separation of the Various Constituents present in the Root and Stem of *Peristrophe bicalyculata*

| CONSTITUENTS      | STEM                      | ROOT                     |
|-------------------|---------------------------|--------------------------|
|                   | % Yield | No. of spots | Rf. Value | % Yield | No. of Spots | Rf Value |
| Alkaloids         | 0.32    | 1            | 0.92      | 0.43    | 3            | 0.58, 0.77, 0.92 |
| Free Coumarins    | 1.1     | 5            | 0.18, 0.35, 0.57, 0.66, 0.76, -- | 0.82, 0.09, 0.14, 0.26, 0.34, 0.49, 0.55, 0.65 |
| Glycosides        | -       | -            | -         | 0.98    | -            | -         |
| Coumarins         | 1.3     | 2            | 0.77, 0.88 | 0.88    | 2            | 0.77, 0.88 |
| Triterpenoid      |          |              |           |         |              |           |
| Saponins          | -       | -            | -         | 4       | 0.20, 0.34, 0.46, 0.56 |
| Free Sugar and    | -       | -            | -         | 0.63    | 1            | 0.45      |
| Potassium Chloride| 1.18    | -            | -         | -       | -            | -         |
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