



ISSN: 0975-833X

RESEARCH ARTICLE

PHYTOCHEMICAL AND PHARMACOGNOSTIC INVESTIGATION ON
OLDENLANDIA CORYMBOSA (L.) Lamk. AN IMPORTANT
HEPATOPROTECTIVE MEDICINAL PLANT

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ARTICLE INFO

Article History:

Received 22nd April, 2015
Received in revised form
17th May, 2015
Accepted 29th June, 2015
Published online 28th July, 2015

Key words:

Prismatic crystals,
Microscopy,
Oldenlandia corymbosa (L.) Lamk,
Pharmacognosy,
Phytochemical analysis.

ABSTRACT

Phytochemical and Pharmacognostic Investigation on *Oldenlandia corymbosa* (L.) Lamk. Of Rubiaceae, has been undertaken. The present investigation involves the macroscopy, microscopy, which showed Prismatic crystals in the transverse section of leaf and stem. Both the surfaces of leaf showed the paracytic type of stomata and the number of stomata were more on the lower surface. Preliminary phytochemical analysis revealed the presence of amino acids, gums, steroids, alkaloids, tannins and phenolic compounds. Microscopic investigations on crude drug powder showed the presence of spiral, pitted, and annular vessels, phloem elements, tannin cells and Prismatic crystals.

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Citation: Gajakosh, A. M. and M. Jayaraj, 2015. "Phytochemical and Pharmacognostic investigation on *Oldenlandia corymbosa* (L.) Lamk. an important Hepatoprotective medicinal plant", *International Journal of Current Research*, 7, (7), 17665-17669.

INTRODUCTION

India has a rich heritage of traditional medicine and people have been using these medicines for many centuries. The traditional system of medicine mainly consists of three major systems namely Ayurveda, Unani and Siddha. In almost every system medicinal plants play a major role and constitute the backbone of traditional medicine. *Oldenlandia corymbosa* (L.) Lamk. belongs to Rubiaceae family, which has wide range of habits including lianas, shrubs, seldom herbs comprising about 450 genera and 6500 species. It is widely distributed. However, most of herbs are distributed in north temperate regions (Sharma, 2009). In china, *Oldenlandia corymbosa* (L.) Lamk. is used in the treatments of viral infections, cancer, symptoms involving toxic heat, acne, boils, skin ailments, appendicitis, eye diseases and bleeding (Chen *et al.*, 1992). The plant is used for venomous bites. It is diuretic, depurative and has digestive and stomachic properties (Rathi *et al.*, 2009). It was used in jaundice and other diseases of liver, heat eruption, violated conditions of pitta, hyperdespia, giddiness, dyspepsia, leprosy, other skin diseases, cough, nervous depression caused by the deranged bile and hepatothy (Sasikumar *et al.*, 2010).

Hepatoprotective property has been also reported against the damage caused by high doses of paracetamol (Sulthana *et al.*, 2010) and galactosamine (Gupta *et al.*, 2012). The relevance of pharmacognosy in standardization of herbal drugs was long been stressed. Many monographs based on pharmacognostic studies have emerged as an aid in the taxonomical and botanical identifications (Kalidass *et al.*, 2009). The process of standardization can be achieved by stepwise pharmacognostic studies reported in *Andrographis paniculata* (Sudhakaran, 2011), *Artimisis pasiflora* Roxb (Modi *et al.*, 2010) and *Argyreia speciosa* Linn (Joghee *et al.*, 2012). These studies will probably help in the identification and authentication of the crude drug. Hence, the present investigation is undertaken to develop pharmacognostic database for *Oldenlandia corymbosa* (L.) Lamk.

MATERIALS AND METHODS

Collection and authentication

Oldenlandia corymbosa (L.) Lamk. was collected from the Karnatak University campus, Dharwad during the months from July to September. The plants were identified by one of the authors and the voucher specimen is kept in the herbarium of

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the department of botany, Karnatak University Dharwad for future references.

Pharmacognostic studies

The collected plant material was shade dried for 8-10 weeks in the laboratory and dried material was coarsely powdered mechanically with the help of grinder and passed through 20 mesh sieve and stored in air tight container for further use. Fresh plant material is also used to study anatomical features. Microscopical characters, physiochemical analysis were carried out using the powder and for macroscopical studies, transverse section of the stem, root and leaf were prepared and stained according to standard methods (Kokate, 2009 and Khandelwal *et al.*, 1950).

Organoleptic analysis

Various sensory parameters such as color, odor, taste, size, shape, texture were studied by the organoleptic evaluation (Kokate, 2009 and Khandelwal *et al.*, 1950).

Microscopic analysis

Microscopic characters were studied by taking hand sections from the fresh plant material and stained according to standard procedures (Kokate, 2009 and Khandelwal, *et al.*, 1950).

Stomatal type, Stomatal number and stomatal index

Stomatal type was determined based on the classification of stomata on the ground of nature and number of subsidiary cells⁶. Stomatal number is the average number of stomata per square millimeter of leaf and stomatal index is calculated by using following equation-

$$SI = \frac{S}{E+S} \times 100$$

Where SI- Stomatal index. S-Stomata, E-number of epidermal cells.

Physicochemical analysis

Physicochemical parameters such as percentage of total ash value, acid insoluble ash, water soluble ash, extractive values were investigated (Kokate, 2009 and Khandelwal, *et al.*, 1950).

Fluorescence analysis

Whole plant powder treated with freshly prepared suitable chemical reagents for 24 hours, then it was subjected to visible light and UV light (short and long wavelength) (Pratt and Chase, 1949. Metcalfe and Chalk, 1950) for fluorescence analysis.

Extraction and phytochemical analysis

Powdered material was subjected to soxhlet extraction, where 25gm of powdered plant material was taken and extracted for 18 hours successively with different solvents like petroleum ether, chloroform, acetone, ethanol and water. Phytochemical

analysis was carried out by standard procedure (Kokate, 2009 and Khandelwal, *et al.*, 1950).

RESULTS

Macroscopic characters

Oldenlandia corymbosa (L.) Lamk. (Fig. 1.) is a procumbent herb with many branches on the stem. Rooting occurs at the nodal region. Leaves are sessile, simple entire, opposite, with interpetiolar stipules. Each peduncles has two flowers that are bisexual, tetra to pentamerous, polysepalous, gamopetalous. 5 stamens are epipetalous, inserted in the corolla tube, alternate with the corolla tube. Ovary is bicarpellary syncarpous and is inferior. Fruit is a capsule.



Fig. 1. Habit of the plant

Organoleptic analysis

Organoleptic analysis indicated the color of the powder was Olive green, smooth in texture, weedy taste and has a characteristic odor.

Microscopic characters

T.S of Stem (Fig. 2A.)

Transverse section of stem showed single layered epidermis and is composed of rectangular cells. Hypodermis is made up of single layer of collenchymatous cells, cortex is made up 3-6 layers of parenchymatous cells with starch granules which is followed by endodermis. Xylem is very prominent and occupies major portion of the stem. Vessels are arranged in radial rows with circular or polygonal in shape. Phloem tissue surrounds the xylem. Centrally placed pith cells contain prismatic crystals of calcium oxalate.

T.S of Root (Fig. 2B.)

Transverse section of root showed the outermost epidermal layer and is cylindrical and composed of tangentially elongated cork cells, Cortex region is found to be reduced, loosely arranged & made up of 3-5 layers of simple parenchymatous cells with simple starch grains and tannin content. Cortex ends with single layer of parenchymatous endodermis. Vascular

bundles are radially arranged and consisted of xylem vessels and fibres. Phloem is situated above the xylem.

T.S of Leaf (Fig. 2C.)

The cells of the epidermis were rectangular in shape, embedded with Prismatic crystals. Only upper epidermis is cuticularized, The mesophyll is dorsiventral and is formed by 1-2 layers of palisade parenchyma and four layers of spongy parenchyma. Small collateral vascular bundles are observed immersed into the mesophyll, The midrib is biconvex; however the convexity is more conspicuous on the abaxial surface. Midrib shows three layers of angular collenchyma underlying the coating system on the adaxial side and 2 strata on the abaxial surface. Six vascular bundles are disposed in an open arc and these structures show a collateral arrangement. stomata are present on both the surfaces, where upper surface has fewer number of stomata than the lower surface and is of paracytic type of stomata (Table 2).

Leaf constants (Fig. 3 A,B.) (Table 2.)

Parameters	Values
Stomatal type	Paracytic
Stomatal number	Upper epidermis 38.3± 0.6 Lower epidermis 96.2 ± 0.14
Stomatal index	Upper epidermis 25.3 ± 0.21 Lower epidermis 16.6 ± 0.18

Average of five readings per microscopic view

Powder microscopy (Fig. C-I.)

Powder microscopic characters showed the presence of spiral, pitted, and annular xylem vessels, phloem elements, prismatic crystals and tannin cells.

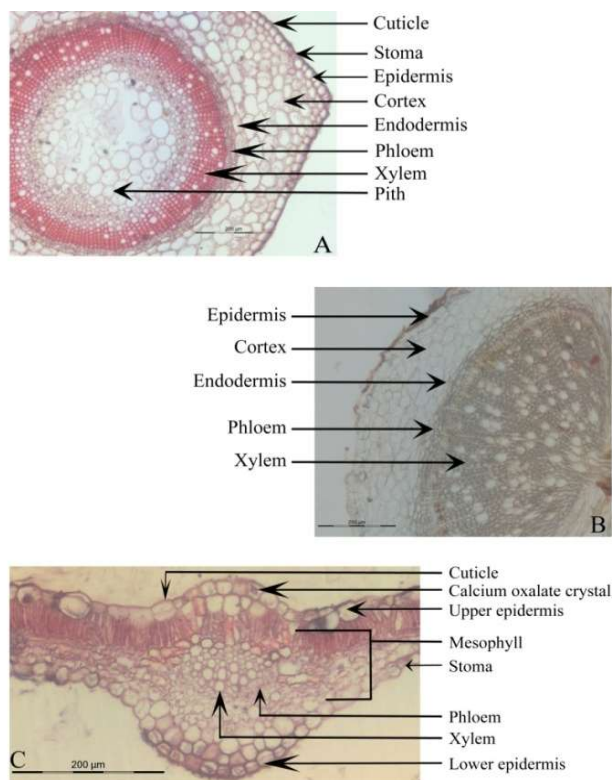


Fig.2. A. T.S of Stem. B. T.S of Root C. T.S of Leaf

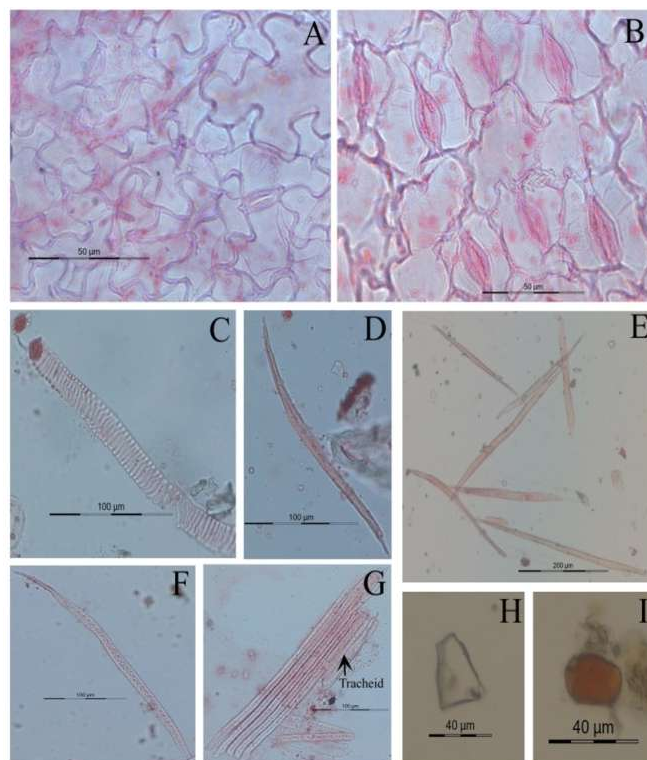


Fig. 3. A. Paracytic type of Stomata on upper surface of leaf, B. Stomata on lower surface of leaf, C. Vessel, D and E. Fibers, F. Spiral Vessel, G. fibers with tracheid. H, Prismatic crystals, I. Tannin cell

DISCUSSION

Standardization is an essential measure of quality, purity and authenticity. Microscopic method is one of the simple and cheapest methods to start with establishing the correct identification of source material. The pharmacognostic work on *Oldenlandia corymbosa* (L.) Lamk. leaf was carried out in methanolic extract showed the absence of the tannins (Mammen *et al.*, 2011). In the present work, macro and micro studies revealed the presence of stomata on both the surfaces of the leaf, stem sections showed the presence of prismatic crystals which is also reported for *Andrographis paniculata* (Sudhakaran, 2011), similarly stem and roots sections showed tannin cells which is also confirmed in powder microscopy (Fig. 2, I). Powdered material was tested with different chemical reagents for fluorescence behavior with different wavelengths of light and the results presented (Table 4). Phytochemical investigations carried out in different solvents showed the presence of sugars, gums, proteins, steroids, alkaloids, tannins and anthraquinones along with other components and the results are presented (Table 5).

Physicochemical analysis (Table 3)

Parameters	Values (w/w)%
Moisture content	7.36 ± 0.29
Ash values	Total ash 9.03 ± 0.25 Sulphated ash 21.61 ± 0.38
Extractive values	Ether soluble 10.04 ± 0.21 Alcohol soluble 8.8 ± 0.17 Water soluble 12.8 ± 0.24

Average of three readings, SD, on dry weight of samples

Fluorescence analysis (Table 4)

Material with Chemical	Day light	UV light (short)	UV light (long)
P + Phloroglucinol: HCl	Brown	Dark green	Black
P + Methanol	Green	Green	Saffron
P + Ethanol	Light green	Green	Saffron
P + Pet. Ether	Brownish green	Green	Brick red
P + Acetone	Green	Green	Brick red
P + Chloroform	Green	Dark green	Brick red
P + 50% H ₂ SO ₄	Green	Dark green	Black
P + 50% HNO ₃	Chrome yellow	Parrot green	Reddish brown
P + 50% HCl	Brown	Dark green	Black
P + 10% NaOH	Light brown	Dark green	Dark brown
P + Ammonia	Brown	Dark green	Purplish black
P + Glacial Acetic acid	Greenish	Dark green	Carnoy's red
P + Picric Acid	Greenish yellow	Chrome yellow	Brown
P + 5% FeCl ₃	Dark green	Green	Black

Phytochemical screening of *O. corymbosa* (Table 5)

Chemical constituents	Chemical test	Petroleum ether	Chloroform	Acetone	Alcohol	water
Sugars	Molish test	+	+	+	+	+
Reducing sugars	Fehling test	-	+	+	+	+
	Benedict test	-	+	+	+	+
	Test for Monosaccharides	-	-	+	+	+
Galactose	Phloroglucinol	-	-	-	-	+
Gums	Fehling test	-	+	+	-	+
	Benedict test	-	+	+	+	+
	Proteins	Biuret test	-	+	-	-
Proteins	Millions test	-	+	-	-	+
	Xanthoprotein test	-	-	-	-	+
	Precipitation test	-	-	-	-	-
	Test solution+ absolute alcohol	-	-	-	-	-
	Test solution + HgCl ₂	-	-	+	-	-
	Test solution + CuSO ₄	-	-	-	-	-
	Test solution + NH ₄ OH	-	-	-	-	-
Steroids	Salkowskimreaction	+	+	+	+	+
Antraquinoneglycosides	Borntrangers test	+	+	-	-	-
	Modified Borntrangers test	+	+	-	-	-
Flavanoides	Shinoda test	-	-	-	-	+
	Sulphuric acid test	-	-	-	+	-
Alkolides	Mayer's test	-	-	-	-	+
	Wagner's test	+	+	+	+	+
Tannins and phenolic compound	Extract + 5% FeCl ₃	-	-	+	-	+
	Extract + Gelatin	-	-	-	-	-
	Extract + acetic acid	-	+	-	-	+
	Extract + Potasium dichromate	-	-	-	-	-
	Extract + Dil Iodine	-	-	-	-	-
	Extract + Dil HNO ₃	+	+	+	+	+
	Extract + 1 drop NH ₄ OH + potasium ferricyanide	-	-	+	+	+
Organic acid	White ppt immediately	-	-	-	-	+
Extract + 5% CaCl	Ppt on shaking	-	-	-	-	-
	Ppt on boiling and cooling	-	-	-	-	-
	Ppt on adding absolute alcohol	+	+	-	-	+

+ Indicate the presence and - indicate the absence of particular compound

Conclusion

The experimental results has shown the presence of various bioactive phytochemicals, these phytochemicals have been reported to produce some therapeutic and physiological effects in the body. This vindicates the usage of these plants traditionally for medicinal purposes. It is also an indication that these plants may serve as raw materials for the development of new drugs.

Acknowledgement

Authors acknowledge the financial assistance of UGC-UPE fellowship and Chairman, P.G. Department of Botany, Karnatak University, Dharwad and USIC for extending facilities.

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