HISTOCHEMICAL STUDIES ON AN IMPORTANT MEDICINAL HERB CURCULIGO ORCHIOIDES GAETRN USING FOLD SCOPE

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Abstract : *Curculigo orchioides* is an important medicinal herb commonly known as Golden eye grass, used in the indigenous system of medicine. It is a small herb with tuberous perennial root system. In Siddha medicine it is called 'Nilappanai kizhangu' in Tamil and Musali in Ayurveda. The root part of the plant is used to cure various ailments. The present investigation deals with the histochemical studies on leaves and roots of *Curculigo orchioides* using fold scope. This study helps in identification and localization of secondary metabolites in the plant parts. Hand sectioned materials were stained with different chemical reagents and observed under the fold scope to identify location of the secondary metabolites. The result of the histochemical studies confirmed the presence of secondary metabolites such as terpenoids, phenol, tannin, lignin, pectin, flavonoids, alkaloids, polysaccharides, starch, mucilage and protein in leaf and root system. Steroids are absent in both leaf and root. Fats and oil are present in leaf but absent in root. The result of present study will be helpful to identify the sources of the plant to be collected for medicinal preparations. Thus, fold scope served as best tool for identification of right plant at the field level without sacrificing the plants for screening of phytochemicals at laboratory level.

Index Terms - *Curculigo orchioides*, foldscope, histochemicals, secondary metabolites

I. INTRODUCTION

Curculigo orchioides belongs to the family Hypoxidaceae (Amaryllidaceae) is one of the hugely useful plants in the indigenous systems of medicine. It is also known as 'Nilapanai kizhangu' in Siddha system and Musali in Ayurvedic systems of medicine. It is a perennial herb with a rosette of sensible with linear lanceolate, membranous leaves and bright yellow colour flowers, closed to ground. It is found in all parts of India from near sea level to 2300 m altitude, especially in rock crevices and laterite soil. It is used as a rejuvenating and aphrodisiac drug. It is also used to improve complexion and used to cure general debility, deafness cough, asthma, impotence, jaundice, piles, diabetes, hemorrhoids, leucorrhoea, menorrhagia, skin, urinary and veneral diseases (Yoganarasimhan, 2000; Patil *et al.*, 2012). In present times, the drug has been extensively studied for its phytochemicals and pharmacological activities. Roots of *C. orchioides* have been reported for their medicinal properties like antioxidant activity (Suri *et al.*, 1999), hepato-protective efficacy (Venukumar and Latha, 2002), antipyretic activity (Pandit *et al.*, 2011) and immune stimulant properties (Lakshmi *et al.*, 2003). In Unani system of medicine, it is used for management of diabetes (Parrotta, 2001).

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. This method have been developed for qualitative and quantitative analysis of virtually all cellular components, including proteins, carbohydrates, lipids, nucleic acids and the range of ionic elements occurring in cell solutions (Gahan, 1984); (Conn, 1989); (Kiernan, 1999). Hence, by considering the medicinal value of the plant, present study was undertaken for developing standards of authenticity to identify the histochemical localization of leaf and tuberous roots of *Curculigo orchioides* using foldscope.

II. MATERIALS AND METHODS

The plant materials were collected from the area of Neelambathy in Coimbatore, Tamil Nadu and identified with the help of Flora of Presidency of Madras. The free hand sections of the leaf and root were treated with the respective stains for histochemical localization. The sections were examined under foldscope and the images were recorded.

2.1. Test for fats and oils (Kadam *et al.*, 2013)

Hand sectioned materials were stained with Sudan-IV (0.5g of Sudan- IV in 100 ml of chloroform) for 20 minutes and rinsed quickly with 50% alcohol and mounted in glycerine. Appearance of red and pink colour indicates the presence of fats and oils.

2.2. Test for Mucilage (Kokate, 2006)

Sections were stained with a drop of freshly prepared Ruthenium red (Dissolve 0.008g of Ruthenium red in 10% of 10ml lead acetate) for about 5 minutes. Pink colour indicates the presence of mucilage.

2.3. Test for Starch (Johansen, 1940)

About 0.2g of Iodide was dissolved in 2% of Potassium iodide solution. A drop of this solution was added to the section. The presence of starch was indicated by conversion of blue to black colour and red to purple colour for newly formed starch.

2.4. Test for Tannin, lignin, phenol and pectin (O Brein *et al.,* 1964)

Sections were stained with an aqueous solution of 0.1% Toludine Blue O stain for a minute and washed with water till there is no excess stain around the sections. A drop of clean water was dropped on the section and observed under microscope. Bluish green colour indicates the presence of tannin, lignin and phenol. Pink to purple colour indicates the presence of pectin.

2.5. Test for protein (Gahan, 1984)

The sectioned material was warmed on a slide with 10% aqueous potassium hydroxide solution and a drop of 1% Copper Sulphate solution was added. Violet colour indicates the presence of protein.

2.6. Test for Sterols (Hardman *et al*., 1972)

25 g of Antimony trichloride was dissolved in 100 ml chloroform. **A** drop of Antimony trichloride solution was added to the transverse section and allowed to stand for about 5 minutes. The presence of sterol was indicated by dark pink (or) Magenta colour.

2.7. Polysaccharide (McManus 1948)

Sections were oxidized in 0.5% to 1% periodic acid for 2-3 minutes followed by treatment with Schiff's reagent for 5 minutes. Sections were then stained for 20 min and observed under microscope. Formation of Dark pink colour indicates the presence of Polysaccharide

2.8. Alkaloids (Evans 1997)

The transverse sections were immersed for a minute in Mayer's reagent and then mounted in glycerine-water (10: 90, v/v) solution. Appearance of grey colour in the cells showed the presence of alkaloids.

2.9. Terpenoids (Harborne, *et al.,* 1984)

Transverse sections were immersed in 2,4-dinitro phenyl hydrazine reagent for a minute and mounted in glycerine-water (10 : 90, v/v) solution. Formation of golden yellow colour indicates the presence of terpenoids.

2.10. Flavonoids (Harborne, *et al.,* 1984)

Sections were stained with 1% of 2-aminoethyl-diphenylborinate in absolute methanol for 2–5 minutes and then mounted in glycerine-water (10: 90, v/v) solution. Light yellow colour indicates the presence of flavonoids.

III. RESULTS AND DISCUSSION

Histochemical studies are the simple techniques used to detect the phytochemical constituents present in the cellular components of the fresh plant materials. Accumulation of secondary metabolites varies between species to species (Momin and Kadam, 2011). In the present investigation, histochemical studies were applied in the fresh leaves and tuberous roots of *C.orchioides* (Fig 1A and B) for detecting various secondary metabolites such as fats& oils, terpenoids, steroid, phenol, tannin, lignin, flavonoids, alkaloids, polysaccharides, alkaloid, polysaccharide, starch, pectin, mucilage and protein. The result of *C.orchioides* leaves showed the presence of all the tested phytochemical constituents in various cellular regions (Table 1). Similarly, tuberous roots are also confirmed the existence of all the secondary metabolites tested, but lipids and sterol are absent (Table 2). In general, fats are widely distributed in the plant body, and they probably occur in small amounts in every plant cell, but in the current observation, fat was found in palisade cells of leaves (Fig 2-A) and absent in root. Similar results were reported in the leaves of *B. monnieri* and *C. asiatica* (Dhale, 2012).

Terpenoids were present only in vascular bundles of leaves (Fig 2-B) and roots (Fig 3-A) of *C. orchioides*. Accumulation of terpene compounds has been reported in many plants i.e. Monoterpenes in labiatae plants are accumulated in the epicuticular cavity of glandular trichomes (Lange and Croteau 1999) while terpenoids of woody plants are secreted into the resin duct. It is reported that terpenoids possess anticancer activity in nature (Franziska *et al.,* 2010).

Tannins are normally abundant in the leaves of many plants, in the testa of seed and in pathological development like galls (Kuster *et al.,* 1956). In the present study, tannins are distributed in the epidermis and vascular bundles of leaves (Fig 2-E) and vascular bundles of root (Fig 3-D). The plants containing tannins are medicinally used as astringents, against diarrhoea, as diuretics, antitumours (Bruyne *et al.,* 1999), antiinflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara *et al.,* 2005).

Flavonoids are the phenolic compounds responsible for the adaptation and defense mechanisms of plants against fungal pathogens, insects and herbivores (Lattanzio *et al.,* 2006). In the present investigation, flavonoids were detected in the vascular bundles of leaves (Fig 2-C) and roots (Fig 3-B). Plants enriched with flavonoid content showed various pharmacological effects including antioxidant, anti-inflammation, anti-platelet, anti-allergic, cytotoxicity, anti cancer and lowering the risk of heart disease (Asif and Khodadadi, 2013). Alkaloids were detected in the epidermis of leaves (Fig 2-D) and vascular bundle of roots (Fig 3-C). They also possess various pharmacological activities such as anticancer, anti-HIV, ant parasitic activity and ant malarial activity (Cunha *et al.,*

2005; Bouayad *et al.,* 2011). Starch and Polysaccharides were also observed in the vascular bundles and mesophyll tissues of leaves (Fig.2-G&H); cortex and vascular bundles in root (Fig.3-F&G).

Protein is present in vascular bundle of leaves (Fig 2-J) and cortex of root (Fig 3-I). Pectin is deposited in the vascular bundle and lower epidermis of leaves (Fig 2- F) and cork cells in root (Fig.3- E). Mucilage is presented in the vascular bundles and mesophyll tissues of leaves and cortex and vascular bundles in root of *C.orchioides*(Fig 2- I & Fig 3- H). Steroids were absent in both leaf and roots of the plant. In contrast, steroids were reported in *C.orchioides* by *Rao et al., (*1978) and Nema and Ramawat (2012). This is due to the influence of age and geographical distribution of the plants.

IV. CONCLUSION

The identification of the chemical compounds of the species and its histochemical localization contribute to enlarge the pharmacognostic knowledge about the species. To inculcate the value of traditional medicine, the biological importance of the plant and its constituents must be elucidated. The histochemical findings in leaves and roots of *C. orchioides* conformed several biologically important compounds such as alkaloids, flavonoids, phenolic compounds, lipids, starch, terpenoids, polysaccharides and protein in the tissues. These informations are essential to identify the quality of the plant before going for herbal formulations. Thus, fold scope served as vital role in early detection of herbal components at the field level without sacrificing the whole plant for identifying the quality of the drug at the laboratory level.

Table 1: Histochemical studies on *C. orchioides* leaf using fold scope

S.No.	Phytochemicals	Reagent/Test	Colour indication	Location
1	Lipid	Sudan IV	Pink	Palisade cells
2	Terpenoid	2,4,Dinitrophenyl hydrazine reagent	Orange yellow	Vascular bundle
3	Steroid	Antimony trichloride solution	-	Absent
4	Phenol	Toludine Blue O stain	Bluish green	Epidermis, Vascular bundle
5	Tannin	Toludine Blue O stain	Bluish green	Epidermis, Vascular bundle
6	Lignin	Toludine Blue O stain	Bluish green	Epidermis,Vascular bundle
7	Flavonoid	Neu's test	Yellow	Vascular bundle
8	Alkaloid	Mayer's reagent	Grey	Epidermis
9	Polysaccharide	Schiff's reagent	Magenta	Mesophyll tissues and Vascular bundles
10	Starch	I-KI solution	Black	Mesophyll tissues
11	Pectin	Toludine Blue O stain	Purple	Vascular bundles and lower epidermal regions
12	Mucilage	Ruthenium red solution	Pink	Mesophyll tissues
13	Protein	Biuret test	Violet	Vascular bundle

Table 2 : Histochemical studies on *C.orchioides* Root using foldscope

S.No.	Phytochemicals	Reagent/Test	Colour indication	Location
1	Lipid	Sudan IV	-	Absent
2	Terpenoid	2,4,Dinitrophenyl hydrazine reagent	Orange yellow	Vascular bundle
3	Steroid	Antimony trichloride solution	-	Absent
4	Phenol	Toludine Blue O stain	Bluish green	Vascular bundle
5	Tannin	Toludine Blue O stain	Bluish green	Vascular bundle
6	Lignin	Toludine Blue O stain	Bluish green	Vascular bundle
7	Flavonoid	Neu's test	Yellow	Vascular bundle
8	Alkaloid	Mayer's reagent	Grey	Vascular bundle
9	Polysaccharide	Schiff's reagent	Magenta	Vascular bundles and cortex
10	Starch	I-KI solution	Black	Cortex
11	Pectin	Toludine Blue O stain	Purple	Cork cells
12	Mucilage	Ruthenium red solution	Pink	Vascular bundle
13	Protein	Biuret test	Violet	Cortex

Figure -1

***C. orchioides* – Habit**

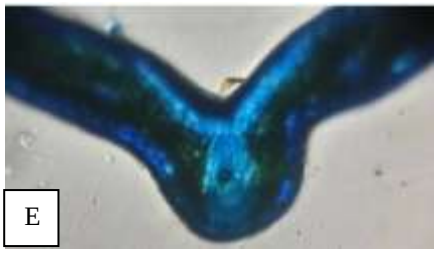
***C. orchioides* – Root**

Figure 2

Fat & Oil (Pink) **Terpenoid (Orange yellow)**

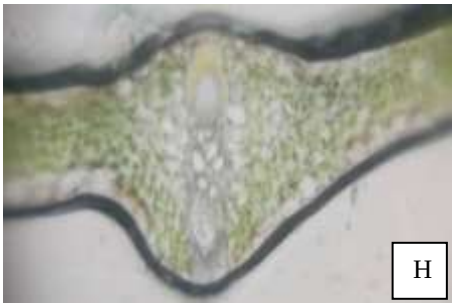
Flavonoid (Yellow) **Alkaloid (Grey)**

Phenol,lignin&tannin (Bluish green) **Pectin (Purple)**

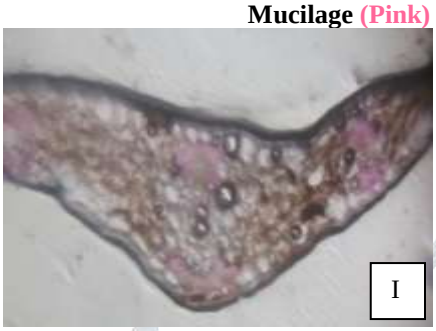


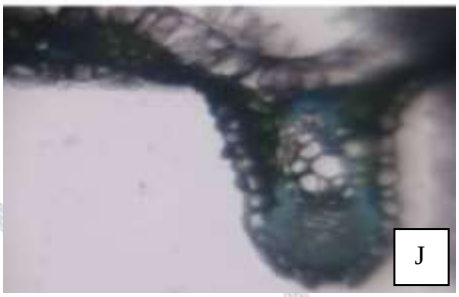
Polysaccharide (Magenta) **Starch (Black)**

Mucilage (Pink)



Protein (Violet)

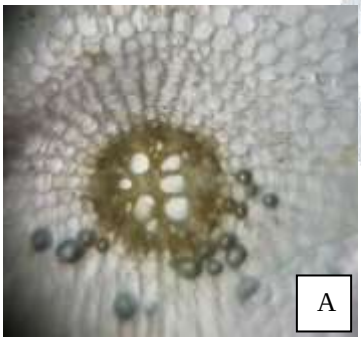


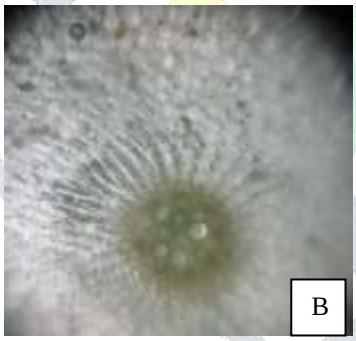


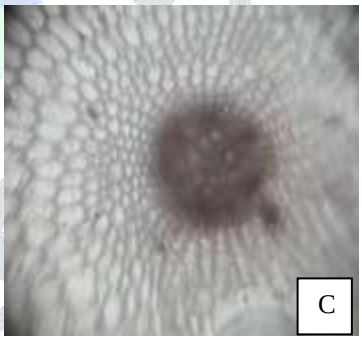
Histochemical studies on *curculigo orchioides* leave (Fold scope view)

Figure 3

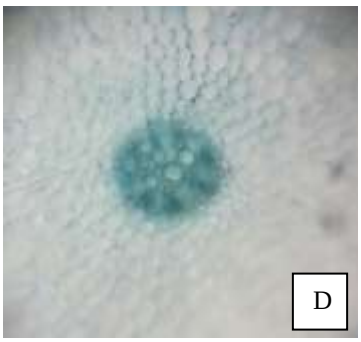
Terpenoid (Orange yellow) **Flavonoid (Yellow)** **Alkaloid (Grey)**

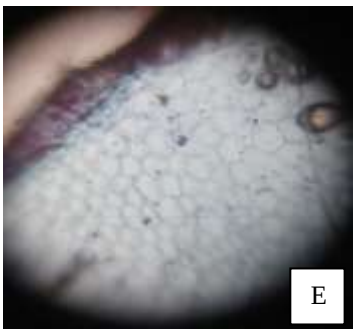


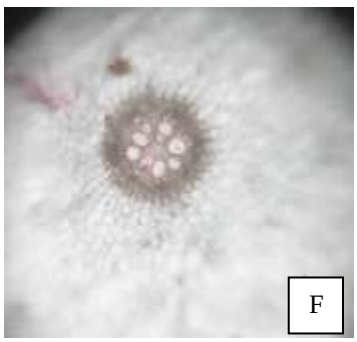




Phenol,lignin&tannin (Bluish green) **Pectin (Purple)** **Polysaccharide (Magenta)**







Starch (Black) **Mucilage (Pink)** **Protein (Violet)**

Histochemical studies on *curculigo orchioides* root (Fold scope view)

V. ACKNOWLEDGMENT

I gratefully acknowledge the DBT -Foldscope Scheme for providing financial assistance to complete this project work.

REFERENCES

[1] Asif, M., and Khodadadi, E., 2013. Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. Journal of Paramedical Sciences, 4 (3): 119- 138.
[2] Bouayad, N., Rharrabe, K., Lamhamdi, M., Nourouti, N.G., Sayah, F., 2011. Dietary effects of harmine, a β-carboline alkaloid, on development, energy reserves and a amylase activity of *Plodia interpunctella* Hübner [Lepidoptera: Pyralidae] Saudi Journal of Biological Sciences, 19(1): 73–80.
[3] Bruyne, T., Pieters, L., Deelstra, H., Vlietinck, A., 1999. Condensed vegetables tannins: biodiversity in structure and biological activities. Biochemical System Ecology, 27: 445–59.
[4] Conn, H.J.,1989. Biological stains: a handbook on the nature and uses of the dyes employed in the biological laboratory. Baltimore:Williams & Wilkins.
[5] Cunha, L.E.V, Fechine, I.M., Guides, D.N., 2005. Protoberberine alkaloids, in: The alkaloids; Chemistry and Biology, 1st edition, ISBN: 0-12-469562-0. Elsevier Academic Press, London, 62.
[6] Dhale, D.A., 2012. Histochemical investigation of two plant species valued as medhya rasayanas. International Journal of Pharma and Bio Sciences, 3(2): 122 129.
[7] Dolara, P., Luceri, C., De Filippo, C., Femia, A.P., Giovannelli, L., Carderni, G., Cecchini, C., Silvi, S., Orpianesi, C., Cresci, A., 2005. Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. Mutation Research, 591: 237–46.
[8] Evans, W.C., 1997. Harcourt Brace and Company, Asia, Singapore, Pharmacology, 226-228.
[9] Franziska, B., Mullauer, Jan, H., Kessler, Jan Paul Medema., 2010. Betulinic Acid, a Natural Compound with Potent AntiCancer Effects, 215-27.
[10] Gahan, P.B.,1984. Plant histochemistry and cytochemistry: an introduc-tion. Orlando: Academic Press.
[11] Harborne, J.B. (1984). Phytochemical methods, London. Chapman and Hall, Ltd. 100-101.
[12] Hardman, R., Safowora, E.A., 1972. Antimony trichloride as a test for steroids especially diosgenin and yamogenin in plant tissues. Stain Technol, 47:205 – 208.
[13] Johansen, D. A., 1940. Plant microtechnique. London New York: McGraw-Hill, 523.
[14] Kadam, P.V., Yadav, K.N., Patel, F.A., Karjikar, F.A. and Patil, M.J., 2013. Pharmacognostic, phytochemical and physicochemical studies of *Piper nigrum* Linn. Fruit (Piperaceae). International Research Journal of Pharmacy, 4(5): 189-193.
[15] Kiernan J.A., 1999. Histological and histochemical methods: theory and practice. Oxford & Boston: Butterworth-Heinemann.
[16] Kokate, C.K., Purohit,A. P. and Gokhale,S.B., 2006. Pharmacognosy. Pune, India Nirali prakashan.
[17] Kuster, E. and Die pflanzenzelle.,1956. 3rd ed., Jene Gustav Fister.
[18] Lakshmi, V., Pandey, K,. Anju Puri, Saxena, R.P., Saxena, K.C.,2003. Immunostimulant principles from *Curculigo orchioides*, J Ethnopharmacol., 89 (2):181-4.
[19] Lange, B. M., Croteau R., 1999. Genetic engineering of essential oil production in mint. Curr. Opin. Plant Biol. 2, 139–144.
[20] Lattanzio, V., Di Venere, D., Linsalata, V., Bertolini, P., Ippolito, A., Salerno, M., 2001. Low Temperature Metabolism of Apple Phenolics and Quiescence of Phlyctaena vagabunda. J Agric Food Chem, 49(12):5817- 5821.
[21] McManus, J.F.A., 1948. Histological and histochemical uses of periodic acid. Stain Technol., 23: 99–108.
[22] Momin, R.K., and Kadam, V.B., 2011. Determination of Lipid and Alkaloid Content in some Medicinal Plants of Genus Sesbania. Journal of Ecobiotechnology, 3(3): 18-21.
[23] Nema, R.K., and Ramawat, K.G., 2012. Isolation and identification of a new molecule from *Curculigo orchioides*

(Hypoxidaceae). International Journal of Pharmacognosy and Phytochemical Research,1(2):24-8.
[24] O' Brien, T.P., Feder, N. and Mc Cull., 1964. M E. Polychromatic Staining of Plant Cell walls by toluidine blue – O. Protoplasma; 59: 364 – 373.
[25] Parrotta, J.A.,2001. Healing Plants of peninsular India, Cabi Publishing, New York, 58-59.
[26] Patil, A.G., Koli, S. P., Patil, D. A, Phatak, A. V. and Nareshchandra, 2012. Pharmacognostic evaluation and hptlc fingerprint profile of *Curculigo orchioides* Gaertn. Roots, International Journal of Pharma and Bio Sciences, 3(3): 101 – 111.
[27] Rao, P.K.V., Ali, A.N. and Reddy, M.N., 1978. Occurence of both saponins and alkaloids from lycorine in *Curculigo orchioides*. Indian J of Pharma Sci., 40:104-5.
[28] Suri, S.S., Jain, S. and Ramawat, K.G.,1999. Platelet regeneration and bulbil formation *in vitro* from leaf and stem explants of *Curculigo orchioides*, an endangered medicinal plant. Scientia Horticulture, 79(1):127-34.
[29] Venukumar, M.R. and Latha, M.S.,2002. Antioxidant activity of *Curculigo orchioides* in Carbon tetrachloride induced hepatopathy in rats. Indian J Clin Biochem. 17(2):80-7.
[30] Yoganarasimhan, S.N., 2000. Medicinal plants of India, Volume 2- Tamilnadu. Bangalore: Cyber Media.