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Microscopic Studies and Preliminary Pharmacognostical Evaluation of Leaves and Roots of *Ophiorrhiza eriantha* Wight

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Abstract: Death and suffering caused by cancer have been a major concern throughout the history of mankind. Extensive research has been undertaken in the plant kingdom to find out an effective drug for the treatment of various cancer diseases. Drugs derived from plants and microorganism plays a major role in the chemotherapy of cancer. Monoterpene indole alkaloid camptothecin (CPT) is a potent anticancer agent first isolated and reported from extracts of the Chinese tree *Camptotheca acuminata* (Nyssaceae). Thereafter camptothecin was isolated from plants of different families mainly *Ophiorrhiza mungos* and *Nothapodytes nimmoniana*. The genus Ophiorrhiza is a potent natural source of camptothecin. The presence of forty-seven species and nine varieties of Ophiorrhiza was reported in the Indian subcontinent. *Ophiorrhiza eriantha* Wight is an erect subshrub species, its anticancer potential and detailed phytochemical analysis are yet to be studied. Our aim is to evaluate the pharmacognostic parameters of leaves and roots of *Ophiorrhiza eriantha* Wight. Our objective is to identify characteristic microscopic features of leaves and roots of the plant and to study the quantitative microscopy, ash value, loss on drying, phytochemical screening, fluorescence analysis, and powder analysis of leaf and roots of *Ophiorrhiza* eriantha Wight. The thickness of the midrib, lamina, leaf margin, and epidermal trichome is measured. It showed the presence of paracytic stomata. Non-glandular epidermal trichomes are common in leaf powder. *Ophiorrhiza eriantha* Wight is a promising anticancer plant; this is a pioneer work to explore the anatomical features of *Ophiorrhiza eriantha* Wight.

Keywords: *Ophiorrhiza Eriantha Wight, Camptothecin, Pharmacognostic Parameters, Powder Analysis, And Fluorescence Analysis.*

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1. INTRODUCTION

From the beginning of life on earth, endless suffering from ailments and diseases also appeared. Death and suffering caused by cancer was a major concern throughout the history of mankind. Despite spending billions of dollars on research, cure or satisfactory treatment is still a challenge in many of the cancer diseases. Plant kingdom served as hope and miraculous cure for his sufferings. Extensive research has been undertaken in the plant kingdom to find an effective drug for treatment of various cancer diseases. More than 60 % of drugs used in current chemotherapy regimens of various types of cancer are derived from plants and microorganism. Monoterpene indole alkaloid camptothecin[1] (CPT) is a potent anticancer agent first isolated and reported from extracts of Chinese tree *Camptotheca acuminata* (Nyssaceae) by M E Wall and M C Wani in 1958.[2] One thousand million US dollars was the estimated turnover of camptothecin derivatives in 2004.[3] The plants belongs to genus Ophiorrhiza shows presence of camptothecin. Forty seven species and nine varieties of Ophiorrhiza reported in Indian subcontinent.[4] Many of them are reported from Southern Western Ghats.[5] Roots of *Ophiorrhiza mungos* is a traditional ayurvedic drug used for treatment of cancer.[6]Tafur S et al studied antiviral and anticancer activity of roots of *Ophiorrhiza mungos.*[7]Basker et al reported preventive activity of Luteolin-7-O-glucoside which isolated from leaves of *Ophiorrhiza mungos* against different cell lines (COLO 320 DM,AGS, MCF-7 and A549) and the result of the study shows that Luteolin-O-glucoside can be used as a potent anticancer drug for colon carcinogenesis.[8] Anti-Snake venom activity of aqueous root extract was reported by Anaswara KS et al. *Ophiorrhiza eriantha* Wight is an erect subshrub belongs to family Rubiaceae. HPTLC based quantification of camptothecin is studied by Satheeshkumar et al.[9] Jose P et al developed a method for micro-propagation of *Ophiorrhiza eriantha* Wight through leaf explant cultures.[10] Our literature review showed that less studies have been done on *Ophiorrhiza eriantha* Wight it. Since *Ophiorrhiza eriantha* Wight is an allied species of potent anticancer plant *Ophiorrhiza* mungos, a comparative anticancer study of *Ophiorrhiza eriantha* Wight and *Ophiorhiza mungos* is relevant and needed. A detailed pharmacognostical study of the plant is not reported so far. In this work we have studied pharmacognostic, microscopic diagnostic features and preliminary pharmacognostical evaluation of leaves and roots of *Ophiorrhiza eriantha* Wight.

2. MATERIALS AND METHODS

2.1 Procurement and Authentication

Whole plant of *Ophiorrhiza eriantha* Wight was collected in December from Palode, Trivandrum district of Kerala, India and authenticated by Prof. Jayaraman, Director, Plant anatomy research centre(PARC),West Tambaram, Chennai, a voucher herbarium specimen prepared and deposited (PARC/2020/4207).

2.2 Preparation and Fixing of Specimen

Healthy plants and normal organs were selected for the study. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml).After 24 hrs. of fixing, the specimens were dehydrated with graded series of Tertiary-Butyl alcohol as per the schedule given by Sass, 1940.Jeffrey's maceration fluid was prepared for clearing leaf.[11] Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.[12]

2.3 Physicochemical Parameters

Physicochemical parameters such as ash value (total ash, acid insoluble ash, water soluble ash and extractive values water soluble extractive, alcohol soluble extractive) were determined.[13] Fluorescence analysis were carried out by treating powder of leaf and root with Methanol, 10% NaOH, dil.NH_3, Con.HNO_3, con.H_2SO_4, Con.H_2SO_4., 10% $FeCl_3$, Acetone + Methanol and 10%Iodine.[14]

2.4 Preliminary Phytochemical Screening

Preliminary phytochemical screening of extracts (Petroleum ether, ethyl acetate, ethanol) of leaves and roots were carried out with standard qualitative procedure.[15] Test for alkaloids, glycosides, phytosterols, saponins, flavonoids, phenolic compounds, tannins were performed**.**

3. RESULTS AND DISCUSSION

3.1 Morphological and Macroscopic Study[16]

Leaves 12-18 cm long, dorsiventral, simple, acute at apex and base, glabrous or sericeous above, pubescent on nerves below, fresh leaves shiny green in colour and light green after drying. It has a bitter taste with a pungent odour[17]. Quantitative microscopy of leaves is done with the help of camera lucida which includes stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio[18]. Results of quantitative analysis is given in table 1.

3.2 Anatomy of The Leaf

In transactional view, the leaf exhibits planoconvex midrib and fairly thick lamina.[19] The midrib is 12.5 µm thick in vertical plane and 18 µm in horizontal plane (figure 1A). There is a short fairly wide adaxial ridge and the abaxial part is wide and thick. The epidermal layer of the midrib thin comprises small but distinct rectangular cells. The ground tissue is homogenous and parenchymatous. The cells are angular compact and thin walled. On the adaxial side, the palisade layer continues in thin, less prominent layer of cells. Beneath the adaxial epidermis is a thick planoconvex zone of thick-walled cells. The ground tissue in the middle of the midrib includes circular thick-walled cells with minute intercellular spaces (figure1A& B). The vascular system consists of wide shallow are of collateral vascular strands. It consists of short parallel compact layers of xylem elements. The xylem cells are angular, narrow, and thick-walled. Along the lower abaxial part of the xylem arc, there are numerous small phloem units located in a discontinuous layer (figure1B).

3.3 Lamina

The lamina is smooth and glabrous. It is bilateral and heteromorphic.[20]The lamina is 170 µm thick (figure 2A &

2B). The adaxial epidermal cells are fairly large thin-walled and squarish in shape. The cells are 30 µm thick. The abaxial epidermal cells are comparatively smaller and squarish in shape. The stomata are abaxial in position. The mesophyll tissue consists of adaxial single horizontal layer of cylindrical palisade cells. The palisade cells are wider in the upper part and narrow in the lower part. They are arranged with wide intercellular spaces (figure 2A & 2B).The spongy mesophyll tissue consists of horizontal layer of vertical pillars with wide air chambers. Small vascular strands are located in the vertical pillars (figure 2A).

3.4 Leaf Margin

The marginal part of the leaf is thin and curved down.[21]The extreme tip is blunt and wide. The epidermal cells and the mesophyll tissues are not altered much. The marginal part of the leaf is 100 µm thick(figure 2C).

3.5 Powder Microscopy ofLeaf

The powder preparation of the leaf shows the following structures when viewed under microscope.[22]

3.6 Adaxial Epidermal Cells

Small fragments of adaxial epidermis are seen in the powder.[23] The epidermal cells are wide with highly wavy anticlinal walls. The epidermis is apostomatic (figure 3A &3B).

3.7 Epidermal Trichomes

Non-glandular epidermal trichomes are common in the powder.[24]The trichomes are long, broad at the base and gradually tapering at the end. The trichomes are multicellular and uniseriate. The cells are vertically rectangular and thick walled. The cells range from 5 to numerous cells in number. The basal part of the trichome is 30 µm thick (figure 4A &4B).

3.8 Abaxial Epidermis

Fragments of abaxial epidermis are frequent in powder. The abaxial epidermal cells are wide with wavy anticlinal cell walls.The epidermis is densely stomatiferous (figure 5A).The stomata are broadly elliptical and the guard cells are 23×30 µm in size.The stomata are surrounded by two narrow lateral subsidiary cells. So the stomata are paracytic type (figure 5B). Summary of epidermal features are given in table 2.

Table 1. Leaf constants of *Ophiorrhiza eriantha* Wight

Leaf contants	Observations	
	Number	Mean±SD
Stomatal number	25-35	28±0.63
Stomatal Index	15-18.5	16±0.38
Vein Islet number	7-10	8±0.55
Vein Termination Number	10-12	9±0.46
Palisade ratio	5-8	6±30.66

Table 1. Shows the results of quantitative microscopy of leaves of Ophiorrhiza eriantha. Stomatal number, Stomatal index, Vein islet number, Vein termination number, palisade ratio is determined using camera lucida.

Table 2. Epidermal characters and their descriptions

Epidermal characters and their descriptions		
Features	Characters	
	Lower epidermis	Upper epidermis
Cells	Squarish and small with wavy anticlinal wall	Squarish and large with wavy anticlinal wall
Stomata	Denslystomatiferous with parasitic stomata	apostomatic
Trichomes	Non-glandular multicellular and uniseriate	Non-glandular multicellular and uniseriate

Table 2.shows the characteristic epidermal features of the leaves of *Ophiorrhiza eriantha* Wight. Cells of upper and lower epidermis are squarish in shape, with wavy anticlinal wall. Lower epidermis is densely stomatiferous with parasitic stomata.Upper epidermis is apostomatic. Both lower and upper epidermis shows the presence of non-glandular multicellular uniseriatetrichomes

3.9 Root

The root is 1.2 mm in diameter. It is circular in cross sectional outline[25]**.**The root consists of broken epidermal layer, thin cortical zone and very thick secondary xylem cylinder surrounded by external phloem (figure 6B).The epidermal layer is not distinct due to frequent breaking of cells. The cortical zone includes two or three layers of fairly wide, compact, angular, parenchyma cells. Phloem elements occur in small discontinuous units. The phloem cells have thick walls and have dark content (figure 7).The secondary xylem cylinder includes radial lines of xylem elements and xylem rays (figure 6B&7). The xylem cells are wide, angular, and thick walled and lignified. In the median zone of the xylem cylinder there is continuous thin ring of small thicker walled xylem cells. It resembles growth ring cylinder. The xylem rays are narrow and straight. The cells are radially oblong, thick walled and lignified (figure 8A&8B).

3.10 Powder Microscopy of Root

In the powder preparation the following elements are observed.[26]

3.11 Epidermal Cells of Root

The epidermal cells are small angular in outline highly thick walled and lignified. The anticlinal walls of the cell are straight(figure 9A).

3.12 Fibres

Xylem fibres are abundant in the powder. There are two types of fibres.[27]Some of the fibres are narrow with thick walls and narrow lumen. The cell walls are thicker and the terminal part of the fibre is thick and pointed. These fibres are called narrow fibres (figure C). The other type of fibre is thick, short with wide lumen. The cell walls are also thick (figure 9A, 9B& 10A).

3.13 Sclereids

Sclereids are occasionally found in the fibre.[28]They are wide, thick walled cells and have numerous bordered pits on their lateral walls. They do not have end wall perforation (figure 10B).

3.14 Vessel Elements

Long narrow vessel elements are common in the powder.[29]The end wall of vessel elements has wide openings. These openings are called end wall perforations.[30]The lateral walls have fibres attached with the cell wall of the vessel element. The vessel elements are 210 µm long and 20µm wide(figure11A&11B).

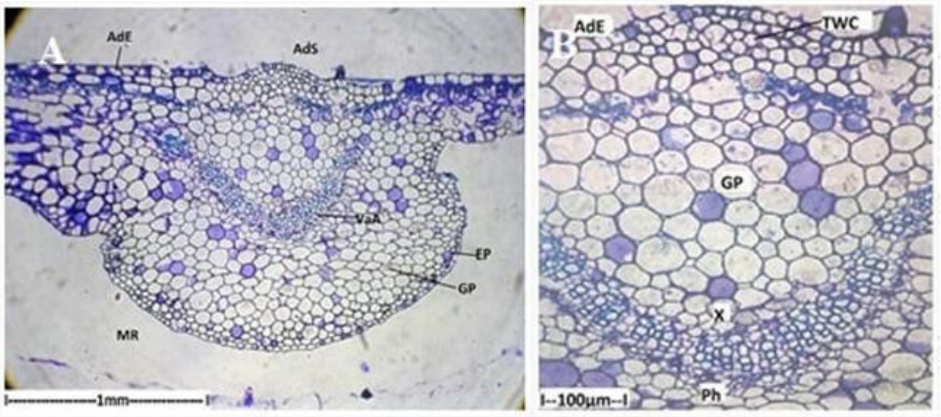


Fig1. A) T S of Leaf through Midrib B) T S of Midrib enlarged

AbE-Abaxial Epidermis; AdS-Adaxial Side; EP- Epidermis; GP-Ground Parenchyma; VaA- Vascular arch; MR- Midrib
AdE-Adaxial Epidermis; TWC- Thick walled cells; GP-Ground Parenchyma; X-Xylem; Ph-Phloem

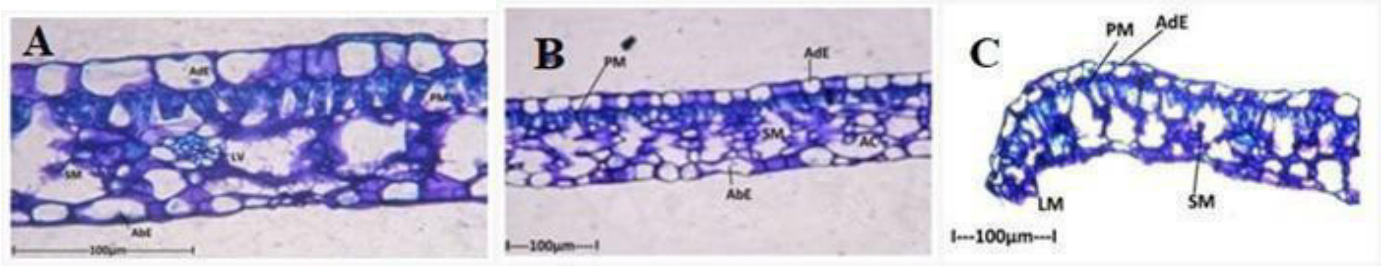


Fig 2.A) T S of Lamina with Lateral Vein B) T S of Lamina C) T S of leaf Margin

AdE-Adaxial Epidermis; LV-Lateral Vein; AbE-Abaxial Epidermis; SM-Spongy Mesophyll; PM- Palisade Mesophyll; AdE-Adaxial Epidermis; SM-Spongy Mesophyll; AC-Air Chamber; AbE-Abaxial Epidermis; PM- Palisade Mesophyll; AdE-Adaxial Epidermis; LM-Leaf Margin; SM-Spongy Mesophyll

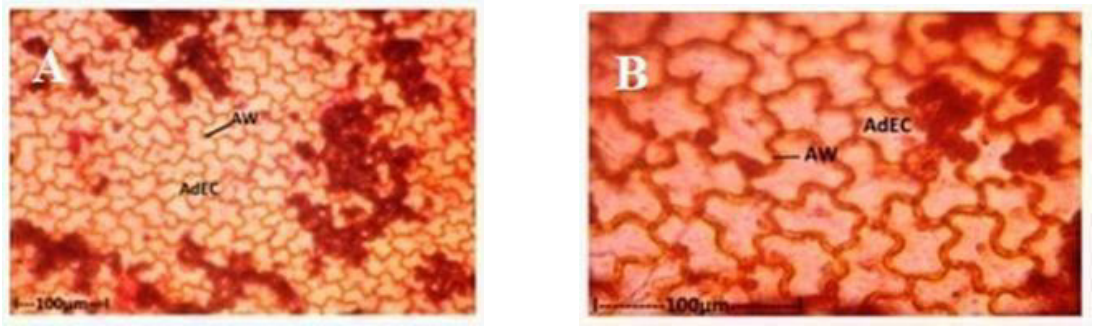


Fig 3.A) Upper Epidermal peeling in Surface view B) Upper Epidermal Peeling Enlarged

AW- Anticlinal Wall; AdEC-Adaxial Epidermal Cells; AdEC-Adaxial Epidermal Cells;AW- Anticlinal Wall

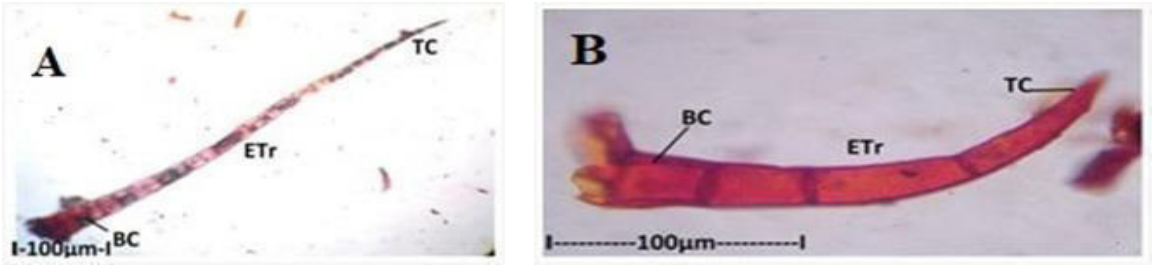


Fig 4.A) Thin long non-glandular trichome B) Short wide non-glandular trichome

BC- Basal Cells; ETr- Epidermal Trichome; TC- Terminal Cells; BC- Basal Cells; ETr- Epidermal Trichome; TC-Terminal Cells

Fig 5.A) Abaxial epidermal cells showing stomata B) A Few stomata enlarged

St-Stoma; AW- Anticlinal Wall; AbEC-Abaxial Epidermal Cells GC- Guard Cells; SC- Subsidiary Cells; St-Stoma; EC- Epidermal Cells;

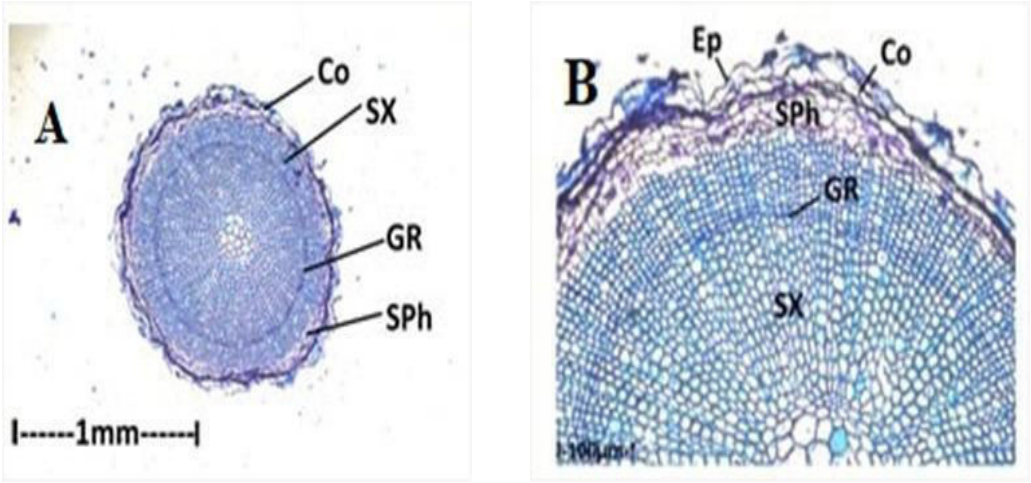
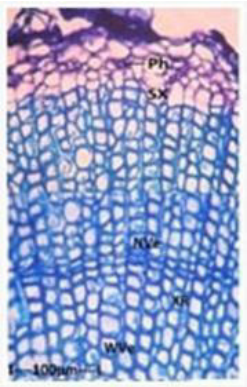
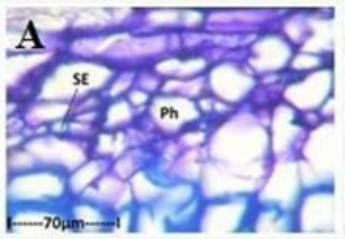


Fig6.A) T S of Root-Entire view B) T S of Root-section enlarged

Co-Cortex; SX-Secondary Xylem; GR-Growth Ring; SPh-Secondary Phloem
EP- Epidermis; Co-Cortex; SPh-Secondary Phloem; GR-Growth Ring; SX-Secondary Xylem;





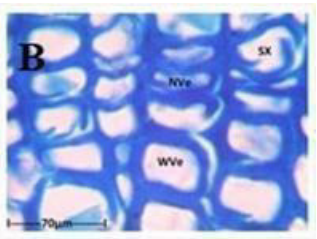


Fig7.T S of Root- Secondary Phloem& Secondary Xylem **Fig 8.A) T S of Phloem** **B) T S of Xylem**

Ph-Phloem; SX-Secondary Xylem; NVe- Narrow vessels; XR- Xylem Ray Narrow vessels. WVe-Wide Vessel
SE-Sieve Elements; Ph-Phloem; NVe- Narrow vessels; WVe-Wide Vessel; SX-Secondary Xylem;

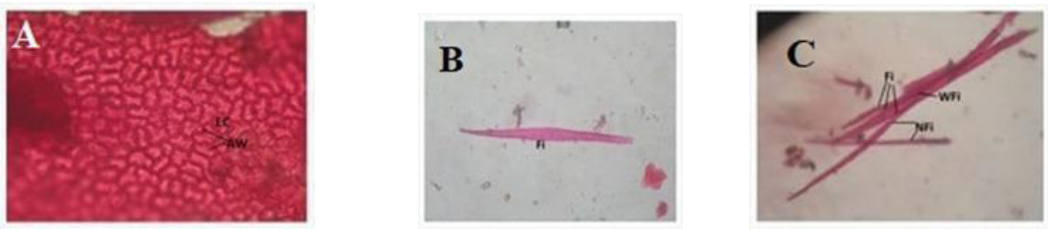


Fig9. A) Epidermal peeling of root B) Wide Fibre C) Wide and narrow fibre

AW- Anticlinal Wall; Fi-Fibres; Fi-Fibres; WFi-Wide Fibre; NFi- Narrow fibres

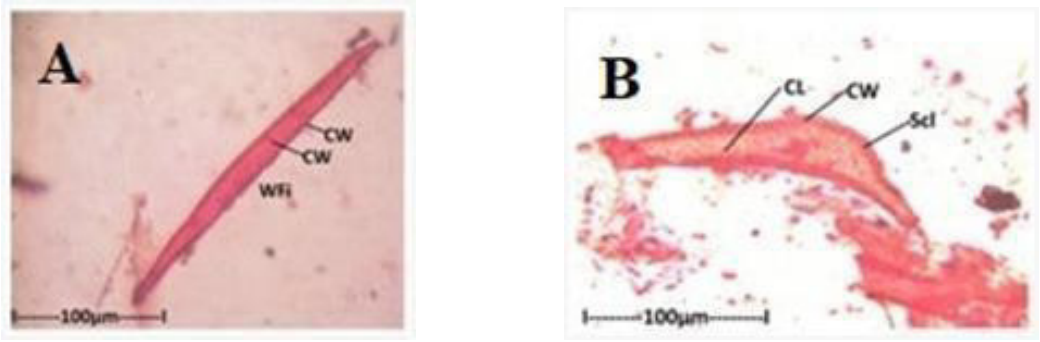


Fig 10.A)wide fibre-Enlarged B) Sclereids

CW-Cell Wall; CW-Cell Wall; WFi-Wide Fibre; CL-Cell lumen; CW-Cell Wall; Scl-Sclereids

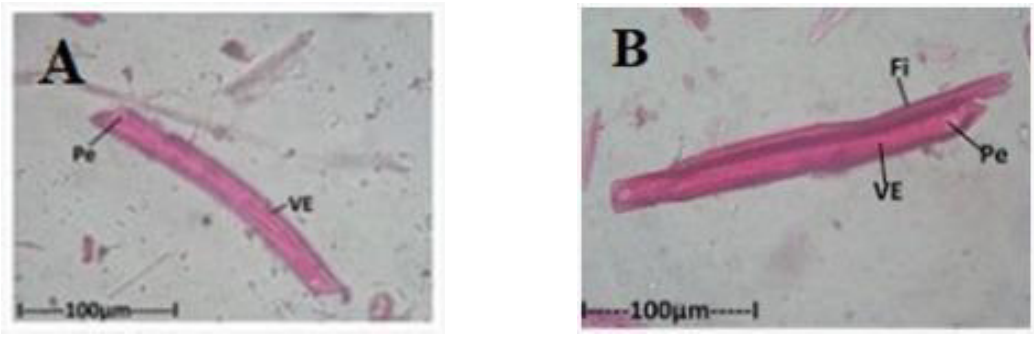


Fig11.A) Vessel Elements- Isolated B)Vessel Elements-isolated

Pe- Perforation at the end wall; VE- Vessel Elements; VE- Vessel Elements; Fi-Fibres; Pe- Perforation at the end wall

3.15 Physicochemical and Phytochemical Studies

Results of the physicochemical and preliminary phytochemical analysis are providing in table 3and 4.Observations of fluorescence analysis of powder after treatment with various reagents are provided in table 5.

Table 3.Physicochemical parameters of leaves and roots of *Ophiorrhiza eriantha* Wight

Physicochemical parameters	Leaves	Roots
Fresh weight (g)	2	2
Dry weight (g)	1.84	1.90
Loss on drying (g)	0.16	0.10
Moisture content(mg/g)	8	5
Total ash (%w/w)	6.34	6.84
Acid insoluble ash (%w/w)	0.55	0.63
Water soluble ash (%w/w)	5.02	5.25
Alcohol soluble extractive (%w/w)	13.2	10.22
Water soluble extractive(%w/w)	21.33	15.34

Table 3.Illustrates the physicochemical parameters of leaves and roots of *Ophiorrhiza eriantha* Wight. Physicochemical parameters are determined as per WHO guidelines. Loss on drying, Total ash value, Acid insoluble ash value, water soluble ash value, alcohol soluble extractive value and water soluble extractive values of both leaves and roots of *Ophiorrhiza eriantha* Wight are determined separately.

Table 4. Phytochemical analysis of *Ophiorrhiza eriantha* Wight leaves and Roots

Chemical Test	Leaves			Roots		
	Pet Ether extract	Ethyl acetate extract	Ethanol extract	Pet Ether extract	Ethyl acetate extract	Ethanol extract
Alkaloids	-	+	+	-	+	+
Carbohydrates&Glycosides	-	-	-	-	-	-
Phytosterols	+	+	+	+	+	+
Fixed oils	+	-	-	+	-	-
Flavanoids	-	-	+	-	-	+
Phenolic compounds	-	-	-	-	-	-
saponins	-	-	-	-	-	-
Tanins	-	-	-	-	-	-
Volatile oils	-	-	-	-	-	-

Table 4.Illustrates the results of preliminary phytochemical screening of leaves and roots of *Ophiorrhiza eriantha* Wight. Powdered roots and aerial part of *Ophiorrhiza eriantha* Wight separately packed in Soxhlet extractor and extracted sequentially with petroleum ether (60-80), Ethyl acetate, and Ethanol. Ethyl acetate and ethanol extract of both leaves and roots shows presence of alkaloids.All of the extracts shows presence of phytosterols. Ethanol extract shows presence of flavanoids.

Table 5.Flourascence analysis

Treatment of Powder	Leaves			Roots		
	UV			UV		
	254nm	365nm	Visible	254nm	365nm	Visible
Powder as such	Greenish brown	Greenish yellow	Greenish yellow	Yellowish brown	Yellowish brown	Yellowish brown
Powder+Methanol	Brownish green	Blackish green	green	Yellowish brown	Bluish brown	Yellowish brown
Powder+10%NaOH	Greenish yellow	Greenish yellow	Greenish yellow	Greenish yellow	Yellowish brown	Yellowish brown
Powder+dil.NH_3	Cow dung green	Greenish yellow	Greenish yellow	Cow dung green	Yellowish brown	Yellowish brown
Powder+Con.HNO_3	Greenish yellow	Greenish yellow	Greenish yellow	Yellowish brown	Yellowish brown	Yellowish brown
Powder+Con.H_2SO_4	Greenish yellow	Greenish yellow	Greenish yellow	Golden brown	Dark brown	Fluorescent yellow
Powder+10%$FeCl_3$	Cow dung green	Greenish yellow	Cow dung green	Cow dung green	Yellowish brown	Yellow brown
Powder+Acetone+Methanol	Greenish yellow	Yellowish brown	Greenish yellow	Yellowish brown	Yellowish brown	Yellow brown
Powder+ 10%Iodine	Dark green	Reddish brown	Dark green	Reddish brown	Reddish brown	Blackish brown

Table 5.Illustrates the results of fluorescence analysis. Powders of both leaves and roots of *Ophiorrhiza eriantha* Wight were treated with methanol,10% NaOH, dil.NH_3, Con.HNO_3,Con.H_2SO_4,10%H_2SO_4,10%$FeCl_3$,,Acetone+Methanol and 10% Iodine. Treated slides were observed under UV (254nm & 365 nm) and visible light.

4. CONCLUSION

Ophiorrhiza eriantha Wight is a promising anticancer plant and our study explored some anatomical features of *Ophiorrhiza eriantha* Wight. The thickness of the midrib, lamina, leaf margin, and epidermal trichome is measured. It showed the presence of paracytic stomata. Non-glandular epidermal trichomes are common in leaf powder. Powder of the root and leaf analyzed separately, identified and measured for each characteristic feature. Future studies can be made for identification, isolation and purification of anticancer phytoconstituents present in *Ophiorrhiza eriantha* Wight and evaluation of the same for in-vitro and in-vivo anticancer activity.

5. ABBREVIATIONS

AbE-Abaxial Epidermis;AbEC-Abaxial Epidermal Cells; AC-Air Chamber;AdE-AdaxialEpidermis;AdEC-Adaxial Epidermal Cells; AdS-Adaxial Side; AW- Anticlinal Wall;BC- Basal Cells; Co-Cortex; CL-Cell lumen;CW-Cell Wall; EC- Epidermal Cells; EP- Epidermis;ETr- Epidermal Trichome; Fi-Fibres; GC-Guard Cells;GP-Ground Parenchyma; GR-Growth Ring LM-Leaf Margin;LV-LateralVein;MR- Midrib;NFi- Narrow fibres ;NVe- Narrow vessels; Pe- Perforation at the end wall; Ph-Phloem;PM- Palisade Mesophyll; SC- Subsidiary Cells;Scl-Sclereids;SE-SieveElements;SM-SpongyMesophyll;St-Stoma; SPh-Secondary Phloem; SX-Secondary Xylem; TC- Terminal Cells; TWC- Thick walled cells;VaA- Vascular arch; VE-

Vessel Elements; WFi-WideFibre;WVe-Wide Vessel; X-Xylem; **XR**- Xylem Ray Narrow vessels.

6. ACKNOWLEDGMENTS

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7. AUTHOR CONTRIBUTION

Dr. Malarkodi Velraj conceptualized and guided the study. Abdul Jaleel K gathered data and prepared original draft. Both authors discussed the methodology and results and contributed to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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