The Journal of Phytopharmacology 2016; 5(6): 225-229
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2016; 5(6): 225-229
November- December

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Pharmacognostical and phytochemical studies on leaves of *Oxalis corniculata* Linn

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ABSTRACT

Oxalis corniculata Linn is an annual herb commonly known as Indian horrel belonging to the family Oxalidaceae. It is found to uses as, anthelminitic, astringent, diuretic, emmenagogue, febrifuge, cardiac disorders, diarrheoa, dysmenorrhoea, hepatic disorders, eye disease, scurvy and toxicity. This research work deals with the Pharmacognostical and Phytochemical studies on leaves of *Oxalis corniculata* Linn microscopic photographs of transverse section (T.S) of Oxalis corniculata should distinct presentation of epidermal trichomes, calcium oxalate crystals and stomata. Standardization of the plant were done with the help of extractive values [alcohol soluble extractive (16%w/w), water soluble extractive (24%w/w)], total ash (15%w/w), acid insoluble ash (1.7%w/w), water soluble ash (1.8%w/w), sulphated ash (0.9%w/w). Pytochemical analysis showed the presence of carbohydrates, glycosides, flavanoids, phenols, tannins, volatile oils, aminoacids and proteins. The dried coarsely powdered *Oxalis corniculata* plant was extracted with ethanol 600C in soxhlet apparatus for 24 hours. Another extract is prepared in cold maceration method for 7 days using water. The both extract was collected and preserved in a vaccum desicator. Fluorescence analysis was carried out for the plant powder and extract. This study various Pharmacognostical and physiochemical parameters for the identification and standardization of the plant material and Phytochemical screening for determination of active constituents.

Keywords: *Oxalis corniculata*, Pharmacognostical standardization, Phytochemical screening.

INTRODUCTION

Oxalis corniculata Mankind has been using plants as therapeutic agents for thousands of years and continue to rely on them or health care needs. According to a WHO estimate, around 80% of the world's inhabitants depend on traditional medicine or their primary health care, majority of whom use plants or their active principles. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases.

Oxalis corniculata (Linn) of family Oxalidaceae, *Oxalis* meaning greek oxys – acid, sharp, sour, referring to the taste of the leaves and stem. *Corniculata* means horn like appendages. The plant is an herb, the branchlets creeping and rooting at the nodes. It is distributed throughout the warmer parts of India, ascending up to an altitude of 3000 m in north –west Himalayas. It is distributed in Mediterranean woods lands and shrubs lands, semi- steppe shrub lands and deserts. This plant mainly used in anodyne, antibacterial, tumors, anti-inflammatory, anti- septic, astringent, carminative and vermiuge. Aqueous extract of the plant shows activity against micrococcus pyrogens var. aures expressed juice of entire plant shows activity against gram positive bacteria [1]. The presence of valuable phytoconstituents such a flavanoids, glycosides, alkaloids and steroidal compounds are present.

MATERIAL & METHODS

Sample collection, processing and storage

Plant was collected from Nilgiris district. The collected specimens was botanically identified and authenticated by Dr. S. Rajan Ph.D., filed botanist, survey of medicinal plants and collection unit, central council for research in homoeopathy, nilgris district. It was identified as *Oxalis corniculata* Linn oxalidiceae family. The leaf *Oxalis corniculata* were shade dried, powdered and was stored in the air tight container and a portion of it was used for the Pharmacognostical and Phytochemical studies.

Macro Morphology

Fresh leaves of *Oxalis corniculata* were collected and different organoleptic features viz shape, size, colour, type, odour, taste were observed. These parameters are considered useful in the qualitative control of the crude drug and evaluated as per standard WHO guidelines.

Microscopy

Leaf of the *Oxalis corniculata* were cut and removed from the plant and fixed in FAA (formalin 5ml + acetic acid 5ml + ethyl alcohol 90ml). After 24 hours of fixing the specimens were dehydrated with tertiary butyl alcohol. The specimens is embedded in the paraffin and sectioned with the help of rotary microtome. The thickness of the sections was 10-12µm, stained with suitable stains like toluidine blue, safranin, fast green and iodine [2,3].

Powder microscopy

Shade dried leaf of *Oxalis corniculata* were powdered well, then the powder was passed through sieve no.60 and used or powder analysis and organoleptic characters such as nature, colour, odour and taste were studied. Powder analysis using various staining reagents viz 1% phloroglucinol in 90% ethanol, conc HCL and N/50 iodine. Slide were observed under the microscope.

Determination of stomatal number and stomatal index

Stomatal number is the number of stomata per sq.mm of epidermis of the leaf. Clear the piece of the leaf (middle part) by boiling with potassium hydroxide solution or alternatively with chlorinated soda. Peel out upper and lower epidermis separately by means of forceps. Keep it on slide and mount in glycerin water. Arrange a camera lucida and drawing board for making the drawing to scale. Draw a square of 1mm by means of stage micrometer. Place the slide with cleared leaf (epidermis) on the stage. Trace the epidermis cell and stomata. Count the number of stomata, also the number epidermal cells in each field. Calculate the stomatal index and stomatal number. Determine the values for upper and lower surface (epidermis) separately.

Quantitative microscopy

Linear measurements of trichomes

Measurement of cell contents of the crude drugs help in their identification, characterization and standardization. So the measurements of trichomes were performed using satage and eye piece micrometer. A small quantity of powdered drug was stained with one drop of each of pholoroglucinol and conc HCL. Mount a little in dilute glycerin. Observe the slide under low power. The trichomes were focussed and the entire length and width of the trichomes was measured by rotating the scale of eye piece micrometer. Each value was multiplied by the calibration factor. Minimum, average and maximum measurements of 20 observations were tabulated.

Fluorescence analysis

Dried powdered were treated with various chemical reagents and various extracts of the *Oxalis corniculata* were exposed to visible, ultraviolet light to study the fluorescence behavior.

Physiochemical parameter

Physiochemical values such as ash value, extractive value, loss on drying and foaming index were determined according to the well established protocols [4].

Preparation of extracts

Hot percolation

The dried coarsely powdered *Oxalis corniculata* plant was extracted with ethanol 60 ℃ in soxhlet apparatus for 24 hours. The solvents were completely recovered from the collected extract under reduced pressure by distillation. The concentrated extract was dried on a water bath and preserved in a vacuum desicator for further studies.

Cold maceration

The dried coarsely powdered *Oxalis corniculata* plant were charged in an aspirator bottle and extracted with water by cold maceration method for 7 days. After decanting and filtering, nearly 80% of the solvent was removed by distillation over boiling water bath and the remaining under reduced pressure. The extract obtained was further dried in vacuum desicator and the extract was used for further studies. The yield of the extracts was noted and phytochemical screening was carried out.

Phytochemical screening

Ethanol and aqueous extracts were subjected to various phytochemical analysis using standard test procedures and reported [5].

RESULTS AND DISCUSSION

Macroscopy

Type – Trifoliate
Colour – Light green
Shape – Obcordate
Margin – Entire
Apex – Emarginate
Base – Symmetrical
Odour – Charateristic
Taste – Pleasantly sour taste
Arrangement – Alternate
Size – Width about 1.4cm to 1.6cm
Length about 0.8cm to 0.9cm
Surface – Upper – Smooth dark green
Lower – Smooth pale green
Texture – Hairy [6-8].

Fig 1: Whole plant

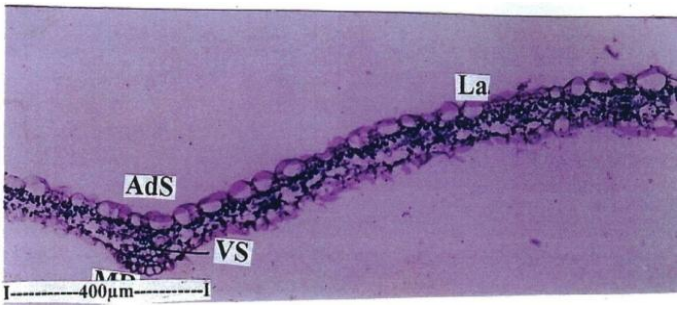
Fig 2: Leaves

Microscopy

The leaf is thin with less prominent midrib. The lamina is uniform in thickness and the lateral venins do no project beneath the surface of the lamina.

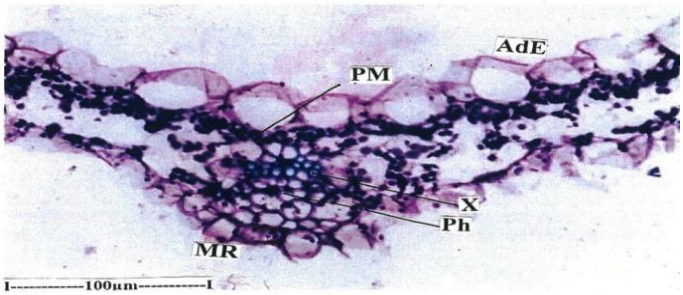
Midrib

The midrib is shallowly concave on the adaxial side and slightly convex on the abaxial side. It is 110µm thick and 70µm wide. The adaxial epidermis is unique and consists of much dilated circular and their outer tangenital walls are slightly projecting; the cells are 40µm thick and 40µm wide. The abaxial epidermis also consists of dilated, thin walled cells similar to the adaxial epidermis (fig 3 & 4).



Ads - Adaxial side, La – Lamina, VS – Vascular bundle, MR – Midrib

Fig 3: T.S of leaflet showing the midrib and lamina



AdE - Adaxial epidermis, Ph- Phloem, X- Xylem, MR- Midrib, PM- Palisade mesophyll

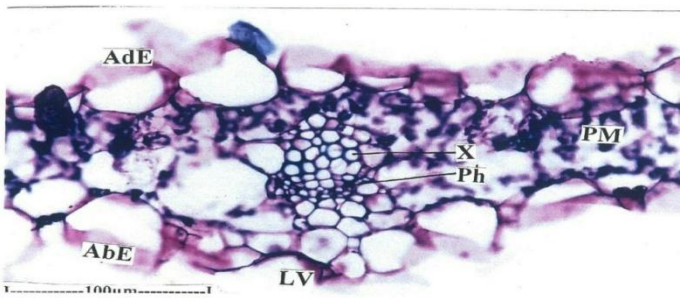
Fig 4: T.S of lealet through midrib (enlarged)

The narrow palisade zone is horizontally transurrent across the adaxial part of the midrib.

The vascular strand is small and collateral and consists of a horizontal tow of 2-cell thick xylum strand and a thin line of phloem elements (fig 4).

Lateral vein

The lateral vein is slightly thicker than the lamina but does not protrude above or below the lamina surace. The vascular strand of the lateral vein consist a group of narrow, angular, thick walled xylum elements and thin sheet of pholem elements. A thin layer of parenchyma cells encircle the vascular strand (fig 5).

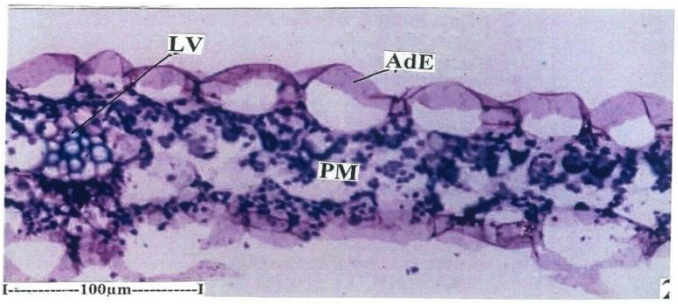


AdE-Adaxial epidermis, AbE-Abaxial epidermis, MR-Midrib, PM-Palisade mesophyll, LV-Lateral vein.

Fig 5: T.S of lealet through lateral vein

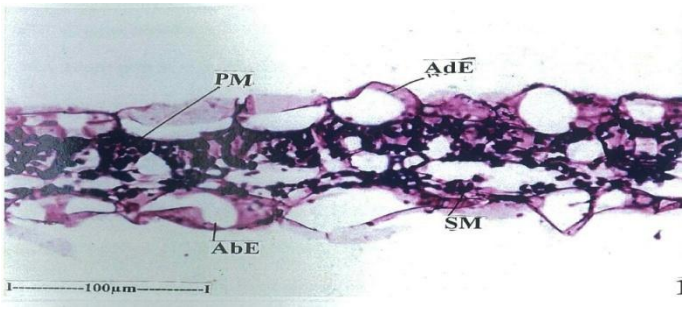
Lamina

The lamina is about 90µm thick. Both adaxial and abaxial epidermal cells are dilated into circular or cylindrical, thin walled cells, the cells are 30-35µm in thickness. The mesophyll is differentiated into a single horizontal band of short, conical palisade cells which are about 30µm in height. The spongy parenchyma cells are two or three layered, they are small spherical and loosely aranged (fig 6, 7 & 8).



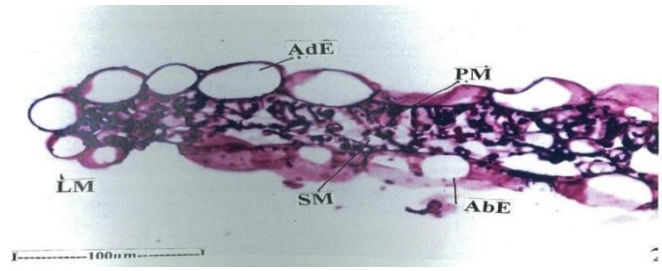
AdE-Adaxial epidermis, PM-Palisade mesophyll, LV-Lateral vein

Fig 6: T.S of Lamina



AdE-Adaxial epidermis, PM-Palisade mesophyll, AbE-Abaxial epidermis, SM- Spongy mesophyll

Fig 7: T.S of Lamina - middle part



AdE-Adaxial epidermis, AbE-Abaxial epidermis, SM- Spongy mesophyll, LM - Lamina margin

Fig 8: T.S of Lamina – marginal part

Powder microscopy

Presence of epidermal trichomes, calcium oxalate crystals and stomata.

Epidermal trichomes

Epidermal trichomes are sparsely seen along the veins. They are unicellular covering unbranched, thick walled lignified and pointed at the tip. The outer surace of the trichome is minutely echinate. The trichomes are upto 1.2mm long and 60µm thick.

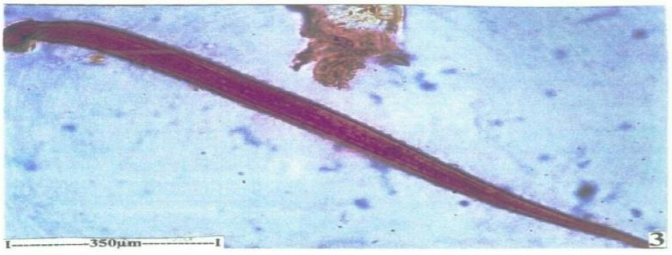
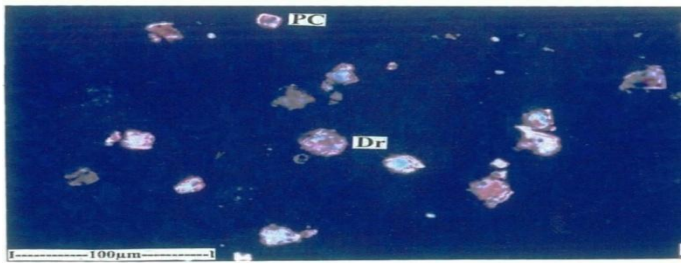


Fig 9: Epidermal trichomes

Calcium oxalate crystals

Calcium oxalate crystals are common in the midrib-ground cells. The crystals are either prismatic type or druses. The crystals are located in ordinary, unspecialized cells. They are solitary and diffuse in distribution. The prismatic crystals are about 10µm, the druses are upto 20µm in diameter.



PC – Prismatic crystals, DR – Druses

Fig 10: Calcium oxalate crystals

Stomata

Stomata are present in lower and upper epidermis. The type of stomata present is anisocytic or cruciferous or unequal – celled stomata. It contains two guard cells which are covered by three subsidiary cells, of which one is markedly smaller than two.

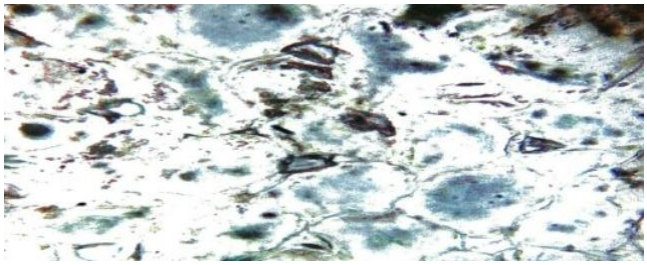


Fig 11: Stomata

Stomatal number and stomatal index-

Determination of stomatal number and stomatal index

1	Stomatal no	4
2	Stomatal index	25

Linear measurements of trichomes

Quantitative microscopy – linear measurement of covering trichomes

PARAMETERS	MINIMUM (µm)	AVERAGE (µm)	MAXIMUM (µm)
Length	313.49	553.37	763.28
Width	10.9	13.35	25.89

Fluorescent analysis

Fluorescence characteristics of the powdered leaves of *Oxalis corniculata.*

Fluorescence characteristics of the powdered of *Oxalis corniculata*

S. NO	TREATMENT	DAY LIGHT	SHORT-UV (254nm)	LONG-UV(365nm)
1	Powder	Greenish brown	Brown	Dark brown
2	Powder + Water	Brown	Yellowish brown	Greenish brown
3	Powder + Ethanol	Greenish brown	Brown	Dark brown
4	Powder + 1N HCL	Yellow	Yellow	Greenish brown
5	Powder + 1N H_2SO_4	Dark brown	Yellowish brown	Dark brown
6	Powder + 1N NaOH	Yellowish brown	Yellowish brown	Bluish brown
7	Powder + 1N alcoholic KOH	Brown	Brown	Dark brown
8	Powder + $FeCl_3$	Greenish brown	Yellowish brown	Dark brown
9	Powder + Acetic acid	Greenish yellow	Yellowish brown	Dark brown
10	Powder + Ammonia	Light green	Yellowish brown	Greenish brown
11	Powder + Iodine	Light yellow	Yellow	Bluish brown

Fluorescence characteristics of the extracts of the leaves of *Oxalis corniculata.*

S. NO	EXTRACTS	DAY LIGHT	SHORT-UV (254nm)	LONG-UV (365nm)
1	Ethanol	Dark green	Dark green	Dark brown
2	Aqueous	Dark brown	Dark brown	Black

The powdered leaves and extracts of *Oxalis corniculata* showed the absence of the fluorescence characters.

Physiochemical parameters

Physiochemical parameters of *Oxais corniculata* leaves

S. NO	PARAMETERS	VALUES (%W/W)
I	**ASH VALUE**	
1.	Total ash	15
2.	Acid insoluble ash	1.7
3.	Water soluble ash	1.8
4.	Sulphated ash	0.9
II	**EXTRACTIVE VALUE**	
1.	Alcohol soluble extractive	24
2.	Water soluble extract	16

Phytochemical analysis

Phytochemical analysis of various extracts

S. NO	TEST OF CONSTITUENTS	ETHANOL	AQUEOUS
1	Carbohydrates	+	+
2	Gums	-	-
3	Mucilage	-	-
4	Proteins	+	+
5	Amino acids	+	+
6	Fats and fixed oils	-	-
7	Steroids	-	-
8	Glycosides	+	-
9	Flavanoids	+	+
10	Alkaloids	-	+
11	Phenols	+	+
12	Tannins	+	+
13	Terpenoids	-	-
14	Quinones	-	-
15	Furans	-	-
16	Resins	-	-
17	Saponins	-	-
18	Volitle oils	+	+

CONCLUSION

In the present work, a medicinally useful plant in the Indian system of medicine, *Oxalis corniculata* was selected. The macroscopic and microscopical study provides a set of diagnostic characters or identification of the plant. The powder microscopy revealed the presence of lignified covering trichomes, prismatic and druses type of calcium oxalate crystals and anisocytic or cruciferous type of stomata. In addition to the above studies, linear measurement of the trichomes and physicochemical constants such as Ash value, Extractive value determined will be useful for identifying this plant and or standardization of the plant by further researchers. Phytochemical studies were carried out. The ethanol extract were prepared by hot percolation. The Aqueous extract was prepared by cold maceration. Preliminary Phytochemical screening aided in identifying the phyoconstituents present in different extracts and the plant powder. It revealed the presence of alkaloids, carbohydrates, flavanoids, glycosides, phenols, proteins, tannins, amino acids and volatile oils. Fluorescence analysis of plant powder and extracts were carried out to detect the fluorescent chromophores.

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HOW TO CITE THIS ARTICLE

Karunanithi S, Rajkishore VB, Pol VG, Sitharathul MM, Abirami. D, Jayshree. Pharmacognostical and phytochemical studies on leaves of *Oxalis corniculata* Linn. J Phytopharmacol 2016;5(6):225-229.