Microscopic Studies and Physicochemical Evaluation of *Antigonon Leptopus* Leaves

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***Abstract*— *Antigonon leptopus* (Polygonaceae) is an evergreen climber native to Mexico. The parts of this plant like seeds, tubers and flowers are consumed as food in several parts of the world. Tea prepared from aerial parts is used as a cold remedy and pain relief. The leaves are used for diabetes, urinary problems, low blood pressure and as a heart tonic. It also has Xanthine oxidase inhibitor and anticancer activity. Though pharmacogostic evaluation of other plant parts of *Antigonon leptopus* is already carried out, no standards are available for the leaf. The present study has been carried out for the authentication and to lay down the standards for the identification of the leaves. Therefore, the fresh leaves and dried powder is used for the microscopical, macroscopical, physicochemical and fluorescence analysis.**

***Keywords*— *Antigonon leptopus*, authentication, micro-morphology, microscopy, physicochemical analysis**

I. INTRODUCTION

Natural products have become very popular and are in huge demand [1]. Exact identity and authentication is required to make sure their purity and safety. Different methods like morphology, anatomy, histochemistry etc. have been used for pharmacognostic standardization of crude drugs[2].

Antigonon leptopus Hook. & Arn. (Polygonaceae) is a native of Mexico. In India, it is distributed in the tropical coastal regions and grows mainly as invasive weed [3]. It is commonly known as 'Coral vine', 'Mexican creeper', 'Bee bush', 'San Miguelito vine' and also 'Anantalata' in India. It is an evergreen climber and can grow up to 40 feet in length. The seeds, tubers and flowers are consumed as food in several parts of the world [4].The aerial portion, including leaves, which is used in the preparation of tea, is used as a cold remedy and pain relief [5]. In Trinidad and Tobago, the leaves are used for diabetes, urinary problems and low blood pressure [6]. It is also used as a heart tonic [7]. Anti-inflammatory, antinociceptive and wound healing activities of the decoction of the flowers are reported [8]. Xanthine oxidase inhibitor activity is shown in aerial parts [9]. The anticancer activity of dichloromethane, methanol and water extracts of the leaves are shown *in vitro* [10]. Results of phytochemical profiling shows that cardiac glycosides,steroids,tannins and terpenoids are present in the leaves. Being a traditional medication used for inflammation and pain, these compounds may potentially find application in pharmaceutical industry.

As the detailed characterization of the leaves is not available, the present study has been carried out for the authentication of the leaves of *Antigonon leptopus* and to solve any related controversial drug identification.

A. Materials and methods

The leaf samples of *Antigonon leptopus* were collected from Altinho, Panaji, Goa, India in the month of January to march 2016.The plant was authenticated by Dr. K Gopalkrishna Bhatt department of botany, Poornaprajna college Udupi, Karnataka and Mr. Dinesh Nayak (Shashyashamala) advisor green belt Mangalore India.

The samples were deposited in Goa College of Pharmacy, department of pharmacognosy, Panaji (voucher no.1532). Fresh samples were used for anatomy and histochemical analysis. Shade dried samples were powered and stored in air tight container at room temperature for further studies. Macroscopic, microscopic, organoleptic and physicochemical studies were carried out[11].The hand sections of fresh leaves were stained with different stains to confer the localization of different components. The leaves were pulverized and separated into fine and coarse powder by sieving through sieve no 40. The powder was stained with different stains to identify different compounds. Stomatal number, stomatal index, vein termination and vein islet number were measured using standard methods [12]. Slides were observed and photographs were taken using Leica DME camera attached with Leica compound microscope. Leica LAS EZ v2.0.0 software was used to analyze the images. All the measurements are taken for 10 times and standard deviation is calculated.

B Results

Fig 1- (a),(b) *Antigonon leptopus* climber, (c) leaf of *Antigonon leptopus*

1) Leaf macroscopy

The plant is a climber with simple and alternate leaves with leathery texture (fig 1a). Leaves are petiolate, cordate to sagittate, margin crenate and shallowly undulate(2–8cm × 4–12 cm). The vein and veinlets are conspicuous at the adaxial surface. The trichomes are present on both the surfaces; abaxial dark green, adaxial paler. The dried leaves are papery and brittle (fig1b,c).

2) Leaf microscopy

Transverse section of the leaf passing through the midrib is broadly convex at adaxial surface and slightly elevated at the abaxial surface. A total of two main meristeles of various sizes embedded in parenchymatous tissue. Lamina is dorsoventral showing single layer of palisade cells and mesophyll (fig 2a).

Detailed TS at midrib showed a single layer of abaxial epidermal cells which are (33.96±0.5 um) rectangular to oval, covered by a thick cuticle; followed by 4–7 layers of collenchymatous cells at the elevation at upper surface as well as 2–3 layers at adaxial surface at midrib. A total of two collateral, conjoint vascular bundles (diameter 340.67 ± 1.8 um) are embedded in parenchymatous ground tissue, upper one is smaller. Parenchymatous cells are embedded with simple and compound starch grains (diameter 7.56 ± 0.8 um). Idioblast cells are often filled with clustered crystals of calcium oxalate (25.3 ± 4.3 um). Adaxial surface contains single layer of epidermis (16.77± 3.3 um) containing thick-walled oval or rectangular cells which are smaller than abaxial epidermis (fig 2b). The lamina shows single layer of elongated palisade cells (44.75± 5 um) under the upper epidermis and 4 to 5 layers mesophyll cells with some air spaces. Multicellular, non-glandular, uniseriate trichomes (30 – 160 um) and few glandular trichomes (10 – 30 um) with multicellular stalk are covering the upper and lower epidermis (fig 2d,e). Trichomes are more on the midrib region as compared to the rest of the lamina.

The lower surface of the leaf contains anisocytic stomata (fig 3a). Surrounding subsidary cells there are polygonal epidermal cells. No stomata are seen in the abaxial surface (fig 3b).

3) Powder microscopy

The observed features were rosette calcium oxalate crystals, prisms, lignified fibres, spiral, reticulate and pitted xylem vessels (fig 3). Parenchyma loaded with starch grains which were both simple and compound. Uniseriate multicellular trichomes having pointed tip, anisocytic stomata with epidermal cells, spongy parenchyma and phloem paranchyma.

4) Pharmacognostic studies

Physicochemical tests such as moisture content, swelling index, foaming index, total ash value, acid insoluble ash, water soluble ash, alcohol soluble extractive and ether soluble extractive were carried out [13]. (TABLE 3). Florescence analysis was done using different reagents under visible light and UV light of wavelength 254nm and 366 nm [14]. (TABLE 1). Fresh leaves were evaluated for stomatal number, epidermal frequency, palisade ratio,vein termination and vein islet number (TABLE 2).

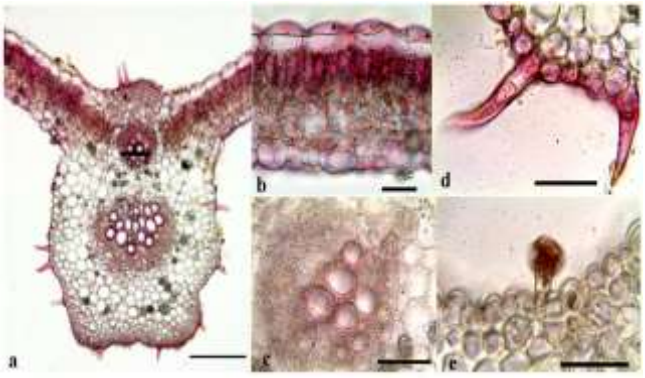

Fig 2- (a) TS of midrib, (b) , (1c) TS of lamina, (c) vascular bundle, (d) uniseriate trichome, (e) glandular trichome

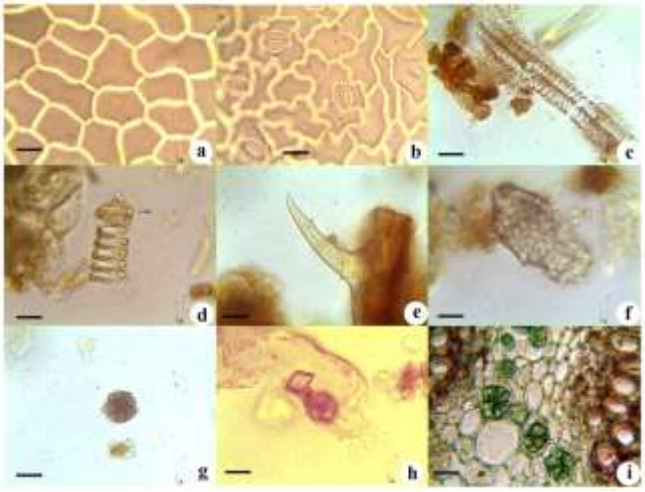

Fig 3- (a) abaxial lamina, (b) Anisocytic stomata, (c),(d) spiral xylem vessel, (e) trichome, (f) compound starch grain, (g) calcium oxalate crystal, (h) prism, (i) cells containing calcium oxalate crystals

TABLE 1
Results of Fluorescence Analysis of Powdered Leaf of *Antigonon Leptopus* [13]

Regents+leaf powder	Short UV (254nm)	Visible	Long UV(366nm)
Powder itself	Light green	dark green	light green
Distilled water	Yellow green	dark green	Fluorescent green
Ethanol	dark green	dark green	Fluorescent green
Methanol	Light green	Light grey	Light green

Toluene	Light green	Light green	Yellow
Iodine solution(5%)	Greenish yellow	Brown	Yellow
Potassium dichromate	Dark green	Fluorescent green	Brown
Ferric chloride(5%)	Dark brown	Fluorescent brown	Green
1N HCl	Dark brown	Grayish	brownish yellow
1N H_2SO_4	Magenta	Magenta	Magenta
Conc. HNO_3	Yellow	Dark brown	Yellow
Picric acid	Light green	Fluorescent green	Greenish yellow
Glacial acetic acid	Yellow	Light orange	Light yellow
HNO_3+NH_3 solution	Yellow	Orange	Orange
NH_3 solution	Green	fluorescent green	Yellow
NaOH	Green	fluorescent green	Orange

TABLE 2
Results of Microscopy of Leaves of *Antigonon leptopus*[16]

Variables	Adaxial surface	Abaxial surface
Stomatal numer	0	300 to 320
Stomatal index	0	17.13
Palisade ratio	3to 5	0
Epidermal cell frequency	1200 to 1302	1450 to 1550
Vein islet numer	9.78 to 15	9.78 to 15
Vein termination numer	12 to 16	12 to 16

TABLE 3

Results of Physicochemical Tests [19] of *Antigonon leptopus* leaf powder

Sr. no.	PHYSICOCHEMICAL TEST	RESULT (%w/w)
1.	Moisture content	11.55 ± 0.95
2.	Swelling Index	0.97 ± 0.05
3.	Foaming index	Less than 100
4.	Total Ash Value	9.5 ± 0.5
5.	Acid Insoluble Ash	1.58 ± 0.152
6.	Water soluble Ash	2.383 ± 0.275
7.	Water Soluble Extractive	5.6 ± 0.52
8.	Alcohol Soluble Extractive	6 ± 0.45
9.	Ether Soluble Extractive	4.5 ± 0.5

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II. CONCLUSIONS

In the present study, the pharmacognostic evaluation of leaves of *Antigonon leptopus* is carried out. The study shows that there are abundant rosette, rhomboidal and prism shaped calcium oxalate crystals. Starch grains are present in both simple and compound form. Stomata are present only on the lower surface and are basically anisocytic but a few are diacytic in nature. Trichomes have taxonomical significance. They are uniseriate muticellular and are abundant. Few glandular trichomes are also seen which are stalked. The other structures in leaf are cuticle, collenchyma and parenchyma.

Evaluation of the extractive values gives idea about exhausted and adulterated drug. Ash values can be used as an aid to find out the impurities and inorganic matter which might be present

Some drugs are not florescent by themselves. They can be made florescent by adding different reagents. Florescence analysis of powdered drug with different reagents under different UV wavelengths are observed and the results are shown in table . it shows that the drug contains active ingredients.

Physicochemical tests are one of the important pharmacognostical parameters. The tests show the degree of purity of the drug.

Presence of two vascular bundles of which the larger one is at the center and smaller one towards the abaxial surface. This is the diagnostic feature of the anatomical part.

Macroscopy and microscopy are useful for morphological and sensory profiles of the drug. These are the quickest and cheapest preliminary methods to lay down the standardization and identification of crude drugs. Therefore the present knowledge can help to establish the genuineness of the crude drug.

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REFERENCES

[1]. Aronoff, S., (1989). Geographic Information Systems: A Management Perspective. Ottawa: WDL Publications.
[2]. Prajna., Srilakshmi, S., Priya, K., Sony.,Swarnalatha, S., Bhojaraju, P., Kanthal, L. K ., Satyavathi, K., (2015). GC-MS analysis and in-vitro cytotoxic activity of methanolic extract of *Antigonon leptopus* Hook. & Arn flowers. International Journal of Pharmaceutical Sciences and Research , 6.7, 3083-3087
[3]. Heinrich, M., (2000). Ethnobotany and its role in drug development. phytother res, 14,479
[4]. Battu, Gangarao; Raju, N. Jaya., (2009).Studies in preliminary phytochemical and antimicrobial activity of *Antigonon leptopus* Hook. & Arn Hook, International Journal of Chemical Sciences, 7(4), 2900-2904.
[5]. Vanisree, Mulabagal; Alexander-Lindo, Ruby L.; DeWitt, David

L.; Nair, Muraleedharan G., (2007).Functional food components of *Antigonon leptopus* Hook. & Arn tea, Food Chemistry 106(2), 487-492

[6]. Mulabagal Vanisree; Alexander-Lindo Ruby L; Dewitt David L; Nair Muraleedharan G., (2011). Health-Beneficial Phenolic Aldehyde in *Antigonon leptopus* Hook. & Arn Tea, Evidence-based complementary and alternative medicine : eCAM, 2011601249

[7]. Lans Cheryl A., (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus, Journal of ethnobiology and ethnomedicine, 245

[8]. Youwei ,Z., Maocheng, D., Yonghong P., (2014).The study of antioxidant activities of extracts from 19 edible flowers.springer plus PMCID:PMC4082252

[9]. Nair, Muraleedharan G.; Vanisree, Mulabagal; Alexander-Lindo, Ruby L.; Dewitt, David L., (2009).Method for inhibiting of COX2 and inflammation with phenolic aldehydes, Int. Appl., WO 2009009056 A1 20090115

[10]. Apaya Maria Karmella L; Chichioco-Hernandez Christine L., (2014). New steroidal saponin from *Antigonon leptopus* Hook. & Arn, Pharmacognosy magazine, 10(Suppl 3), S501-5

[11]. Wongwattanasathien O; Kangsadalampai K; Tongyonk L., (2010).Antimutagenicity of some flowers grown in Thailand, Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association , 48(4), 1045-51

[12]. Gurav,Shailendra S.,Gurav,Nilambari S., (2014).Indian Herbal Drug Microscopy.Springer

[13]. Wallis, T.E., (1984). Practical Pharmacognosy, J. & A. Churchill Ltd., London. (Vth Ed.),

[14]. Kokate, C.K., Purohit, A.P. and Gokhale, S.B., (1999).Pharmacognosy, Nirali Prakashan, XII ed.

[15]. Anonymous.,(1998).Macroscopic and microscopic Examination: Quality Control Methods for Medicinal Plant Materials, WHO, Geneva.

[16]. *Anonymous*., (2007). Indian Pharmacopoeia, Part- II (Formulations), Vol. I, First edition, Government of India. Ministry of Health and Family Welfare, The Controller of Publications, 78,191

[17]. Anonymous., (2001). The Ayurvedic Pharmacopoeia of India, Government of India, Ministery of Health & Family Welfare, Published by The Controller of Publications, Civil Lines, New Delhi, Vol.I.

[18]. Anonymous., (2007). Quality control methods medicinal plants material, World Health Organization, Geneva.

[19]. B.S.Nayak and K.N.Patel. pharmacognostic studies of the jatropha curcas leaves International Journal of pharmtech Research CODEN (USA): IJPRIF ISSN : 0974-4304 Vol.2, No.1, pp 140-143,

[20]. Khandelwal, K.R., (1998).Practical Pharmacognosy, Nirali Prakashan, 5th ed.

[21]. Khedkar, P.V., (2000).Pharmacognostic studies in some marketed crude drugs, A thesis submitted to University of Mumbai for the degree of M.Sc.

[22]. Trease, G.E. and W.C. Evans., (1996). A textbook of pharmacognosy. 14 Ed. Bailliere Tindall Ltd. London