

A STUDY TO EXPLORE THE PHARMACOGNOSTIC AND PHYTOCHEMICAL SCREENING OF *ARTEMISIA NILAGIRICA* LEAVES FOUND IN NILGIRIS DISTRICT OF TAMIL NADU

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Received: 30 Jun 2012, Revised and Accepted: 09 Aug 2012

ABSTRACT

Artemisia Nilagirica is an aromatic, herbaceous perennial plant belongs to *Asteraceae* family used in traditional medicine for the treatment of various ailments. The primary aim of the present study was to explore the pharmacognostic and phytochemical screening of *Artemisia Nilagirica* leaves found in Nilgiris district of Tamil Nadu. Fluorescent behaviour of the leaf and phytochemical studies were performed. Microscopical studies revealed the presence of thick midrib, thin lamina, anomocytic stomata and glandular trichomes. Phytochemical studies revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, terpenoids, steroids, saponins, essential oils, tannins and phenol in various extracts. These pharmacognostic and phytochemical characterization data would be helpful in authentication of raw material or crude drug of *Artemisia Nilagirica* leaves found in Nilgiris district of Tamil Nadu.

Keywords: *Artemisia Nilagirica*, *Artemisia Vulgaris* Linn, Chrysanthemum Weed, Indian Worm Wood, Makkippu.

INTRODUCTION

Artemisia Nilagirica is popularly known as 'Makkipu' and 'Masipattiri' in Tamil. It is an aromatic, herbaceous perennial plant belongs to *Asteraceae* family, which grows up to 150 cm on nitrogenous soils in hilly districts of India. *Artemisia Nilagirica* is also indigenous to Europe, Asia, northern Africa, Alaska and North America. The plant has red-purplish angular grooved stem, small yellow flowers (between July to September) and the leaves are 5-20 cm long, pinnate, smooth, dark green on top and pale green with small white hairs on the bottom (Fig.1 and 2). *Artemisia Nilagirica* is used in traditional medicine for the treatment of various ailments such as poor appetite, indigestion, constipation, travel sickness, parasitic infection, irregular menstruation, menstrual pain, cramp, cold, epilepsy, typhoid, tuberculosis, urinary calculi, colic, fever, asthma, bronchitis, sciatica, flatulence, anaemia, insomnia, gout, depression, nervousness, hysteria, measles, bruise, chilblain, leprosy, pruritus, malaria, fungal infection, leishmaniasis and cancer. This herb has also been used as antiseptic, antioxidant, anthelmintic, expectorant, diuretic, diaphoretic, cholagogue and insect repellent. However, the secondary metabolites such as flavonoids, terpenoids, saponins, polysaccharides and components present in the essential oil are responsible for the therapeutic properties of *Artemisia Nilagirica*.¹⁻¹⁷ The primary aim of the study was to explore the pharmacognostic and phytochemical screening of *Artemisia Nilagirica* leaves found in Nilgiris district of Tamil Nadu.



Fig. 1: Whole plant of *Artemisia Nilagirica*

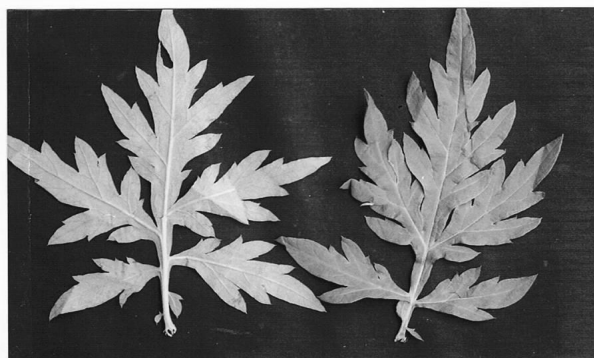


Fig. 2: External feature of the *Artemisia Nilagirica* leaf

MATERIALS AND METHODS

Collection and authentication of the plant material

Fresh whole plant of *Artemisia Nilagirica* was collected from Nilgiris district of Tamil Nadu in the month of September 2010. The plant was identified and authenticated at 'The Rapinat Herbarium and Centre for Molecular Systematics' of St. Joseph's College at Tiruchirappalli, Tamil Nadu.

Reagents and chemicals

The chemicals and solvents used for the pharmacognostic and phytochemical screening of *Artemisia Nilagirica* leaves were of analytical grade.

Microscopic analysis

Healthy normal leaves of *Artemisia Nilagirica* were cleaned using deionized water and divided in to two parts. First part of cleaned leaves was preserved in Formaldehyde-Acetic acid-Alcohol solution (5 ml of Formalin, 5ml of Glacial acetic acid and 90 ml of 70% Ethanol) for further use in anatomical studies. Second part of cleaned leaves was shade dried, powdered using mechanical grinder and packed separately in an air tight container for powder microscopy and phytochemical studies.

Microscopic investigations¹⁸⁻²⁵

Sections (10-12 μ m thick) and surface preparations were used for microscopy according to the methods reported earlier with slight modifications. Photographic images were taken using Nikon lab photo 2 microscopic unit.

Phytochemical analysis²⁵⁻³²

50 gm of the powdered *Artemisia Nilagirica* leaves were extracted using different solvents such as petroleum ether, n-hexane, diethyl ether, benzene, ethylene dichloride, ethanol and water successively by hot percolation method. The extracted material was used for the phytochemical analysis. 1 gm of various extracts of *Artemisia Nilagirica* leaves were dissolved in 100 ml of appropriate solvent (i.e. mother solvent) separately to prepare a stock solution of 1% w/v and then subjected to phytochemical screening using following methods.

Test for Alkaloids**Dragendorff's test**

1 ml of Dragendorff's reagent (Potassium Bismuth Iodide Solution) was added to each 1 ml stock solution of various extracts separately. Formation of an orange-red precipitate indicates the presence of alkaloids.

Mayer's test

1 ml of Mayer's reagent (Potassium Mercuric Iodide Solution) was added to each 1 ml stock solution of various extracts separately. Formation of whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Test for Amino acids**Ninhydrin Test**

3 drops of 5% ninhydrin solution was added to each 3 ml stock solution of various extracts separately in a test tube and heated in boiling water bath for 10 minutes. Formation of purple/bluish colour indicates the presence of amino acids.

Cysteine test

2 ml of 40% w/v sodium hydroxide and 2 drops of 10% w/v lead acetate solution was added to 5 ml stock solution of various extracts separately in a test tube and heated in boiling water bath for few minutes. Formation of black precipitate indicates the presence of amino acids.

Test for carbohydrates**Barfoed's test**

1 ml of Barfoed's reagent was added to each 2 ml stock solution of various extracts separately in a test tube and heated in boiling water bath. Formation of reddish brown precipitate indicates the presence of carbohydrates.

Fehling's test

1 ml of Fehling's solution A and B were added to each 1 ml stock solution of various extracts separately in a test tube and heated in boiling water bath. Formation of brick red precipitate indicates the presence of sugar.

Benedict's test

5 ml of Benedict's reagent was added to each 1 ml stock solution of various extracts separately in a test tube and heated in boiling water bath for 2 minutes. Formation of red precipitate indicates the presence of sugar.

Test for flavonoid**Alkaline reagent test**

Few drops of dilute ammonia were added to each 1 ml stock solution of various extracts separately, followed by addition of concentrated HCl. Formation of yellow colour indicates the presence of flavonoids.

Zinc hydrochloride test

Few drops of stock solution of various extracts were added to zinc dust separately, followed by addition of concentrated HCl. Formation of red colour indicates the presence of flavonoids.

Aluminium test

Few drops of 1% aluminium solution were added to each 1 ml stock solution of various extracts separately. Formation of yellow colour indicates the presence of flavonoids.

Test for glycosides**Keller-Killiani test**

5 ml stock solution of various extracts was added to a mixture of 5 ml of water and 2 ml of glacial acetic acid containing one drop of ferric chloride solution, followed by addition of 1 ml of concentrated sulphuric acid. Formation of a brown ring at the interface followed by formation of a violet ring below the brown ring and formation of greenish ring in the acetic acid layer just above the brown ring and gradually spread throughout layer indicates the presence of glycosides.

Baljet test

Few drops of picric acid were added to each 1 ml stock solution of various extracts separately. Formation of orange colour indicates the presence of glycosides.

Test for tannins and phenol**Lead acetate test**

About 2 ml of basic lead acetate solution was added to each 2 ml stock solution of various extracts separately. Formation of white precipitate indicates the presence of tannins.

Ferric chloride test

About 1 ml of ferric chloride solution was added to each 1 ml stock solution of various extracts separately. Formation of blue black or brownish green colour indicates the presence of tannins.

Ammonia test

About 2 ml of aqueous ammonia solution was added to each 2 ml stock solution of various extracts separately. Formation of a deep green colour indicates the presence of tannins.

Test for terpenoids and steroids**Salkowski test**

0.5 gm of various extracts was added to 2 ml of chloroform, followed by addition of 3 ml of concentrated sulphuric acid. Formation of a reddish brown colour in the interface indicates the presence of terpenoids.

Liebermann's test

10 ml of acetic anhydride was added to each 10 ml stock solution of various extracts separately in a test tube, heated and cooled followed by addition of few drops of concentrated sulphuric acid along the sides of the test tube. Formation of blue colour indicates the presence of steroids.

Test for Saponins

2 gm of various extracts was boiled in 20 ml of distilled water in a water bath and filtered. To the 10 ml of filtrate, 5 ml of distilled water was added and shaken vigorously until the formation of froth. About 3 drops of olive oil was added to the froth and again shaken vigorously. Formation of emulsion indicates the presence of saponins.

Test for lignin

2 ml stock solution of various extracts was treated with saffranine solution. Formation of pink colour indicates the presence of lignin.

Test for essential oil

A drop of Sudan red 3rd reagent was added to the thick section of various extract on a glass slide, washed with 50% alcohol after 2 mins and mounted in glycerine. Appearance of red coloured oil globule under microscope indicates the presence essential oil.

RESULTS AND DISCUSSION

Microscopical characteristics of *Artemisia Nilagirica* leaves

Artemisia Nilagirica leaves consists of a thick midrib and thin lamina (Fig.3). The midrib has short wide adaxial part forming a conical hump. The abaxial part was wide, thick and slightly lobed. The epidermal layer was thin, comprising narrow squanish cells. The ground tissue of the adaxial cone includes three or four layers of collenchyma cells. Abaxial part of the ground tissue consists of large, circular or angular compact thin walled parenchyma cells.

The vascular system consists of a large, centrally placed main bundle and two small, less prominent accessory lateral bundles. The main and accessory bundles were collateral with several short parallel lines of xylem elements and a thick abaxial arc of phloem elements. The xylem elements were angular, wide and thick walled. The lateral bundles have small cluster of xylem and phloem elements. The midrib was 950 μm thick, the adaxial cone was 350 μm wide and the abaxial part was 1 mm wide. The median larger vascular bundle was 450 μm wide and the small lateral bundles were 50 μm thick.

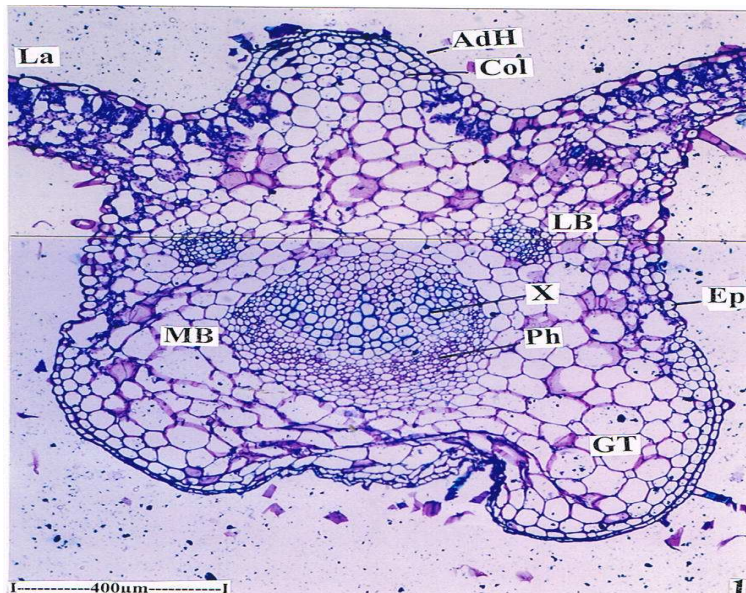


Fig. 3: Transverse section of leaf through midrib and lamina

(AdH: Adaxial Hump; Col: Collenchyma; Ep: Epidermis; La: Lamina; GT: Ground Tissue; MB: Median Bundle; LB: Lateral Bundle; Ph: Phloem; X: Xylem)

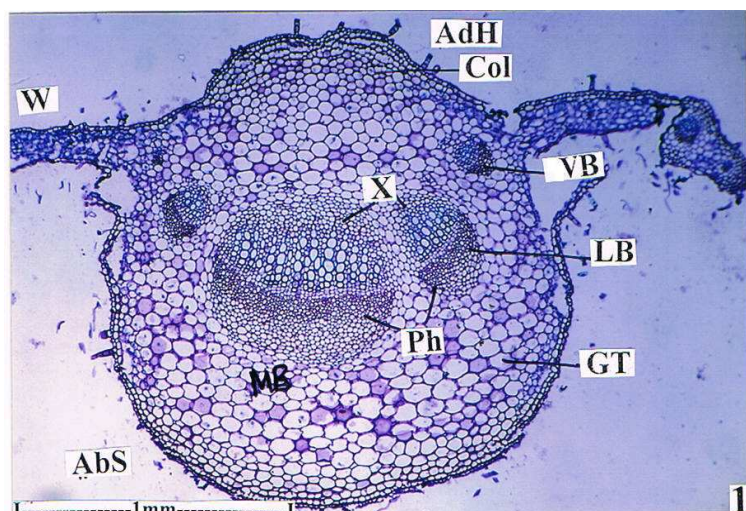


Fig. 4: Transverse section of petiole with wings

(Abs: Abaxial side; MB: Median Bundle; VB: Vascular Bundle; Ph: Phloem; GT: Ground Tissue; X: Xylem; Col: Collenchyma; AdH: Adaxial Hump; W: Petiole with wings)

Lateral Veins

The lateral vein (Fig.4) consists of broad conical abaxial part and slightly convex adaxial part. The veins were 450 μm thick and 300 μm wide. The epidermal layer was made up of circular or barrel shaped dilated cells. The abaxial conical part consists of about three layers of collenchyma. There was a single, circular, prominent vascular bundle consisting of two or three short rows of xylem elements and a small cluster of phloem elements. The vascular

bundle was surrounded by a single layer of dilated by-line bundle sheath cells.

Lamina

The lamina (Fig.3 and 5) was dorsiventral, thin and 150 μm thick. The adaxial epidermal layer was fairly thick with spindle shaped cells. The adaxial epidermis was thin with narrow cylindrical cells. The mesophyll tissue was differentiated in to adaxial, single layer of palisade cells and abaxial zone of three layers of lobed aerenchymatous spongy parenchyma cells.

Petiole

The petiole (Fig.4, 6, 7 & 8) was thick (1.9 mm) and prominent. It consists of a wide and low adaxial hump, wide and thick semicircular abaxial part. The adaxial hump was 900 μm wide and the lower part was 1.6 mm wide. The petiole consists of thin intact epidermal layer of small squarish cells. The ground tissue was differentiated into outer, 3-5 layers of collenchyma cells, remaining region were parenchymatous in nature. The vascular system was multistrated avc of four vascular bundles. Out of the four bundles, the median one was the largest, measuring 700 μm wide and other three bundles were small and lateral in position. The vascular bundles have several fairly long parallel chains of xylem elements with narrow gaps. The xylem elements were wide, angular and thick walled. The metaxylem cells were 25 μm wide. Phloem occurs in thick abaxial band which includes small nests of scattered sieve elements and wide parenchyma cells. The lower end of the vascular bundles have wide

and thick cap of sclerenchyma cells which were thin walled, wide and angular.

Epidermal cells and stomata

The epidermal cells (Fig.9 and 10) were wide, wavy and thick anticlinal walls. The epidermal cells assume amoeboid outline. The stomata have large guard cells (20x30 μm in size) with wide slit like aperture. The stomata were anomocytic type and no distinct subsidiary cells were evident.

Venation pattern

The lamina exhibits dense reticulate venation. The veins were different in thickness, wide and distinct vein-islets (Fig.11). The islets were rectangular to square shaped. Vein-terminations were seen in most of the islets. These terminations were simple (unbranched), long, slender and undulate (Fig.12).

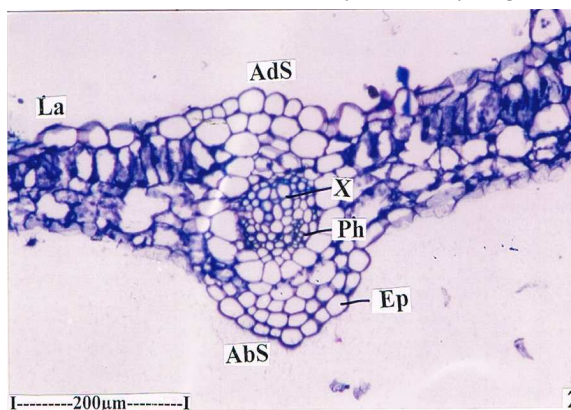


Fig. 5: Transverse section of lamina through lateral vein

(AbS: Abaxial side; AdS: Adaxial side; Ep: Epidermis; La: Lamina; Ph- Phloem; X: Xylem)

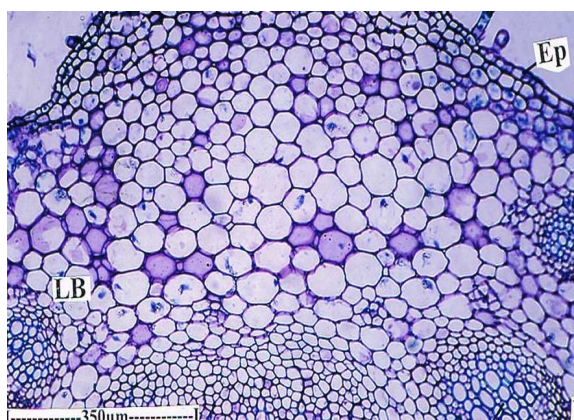


Fig. 6: Transverse of petiole (Upper sector)

(Ep: Epidermis; LB: Lateral Bundle)



Fig. 7: Transverse of petiole (Middle sector)

(MB: Median Bundle; Ph: Phloem; X: Xylem)

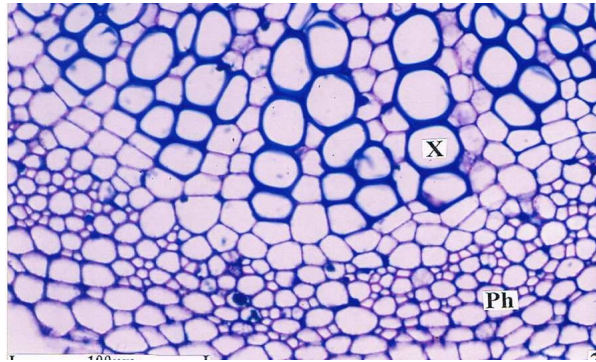


Fig. 8: Transverse of petiole (Lower sector)
(Ph: Phloem; X: Xylem)

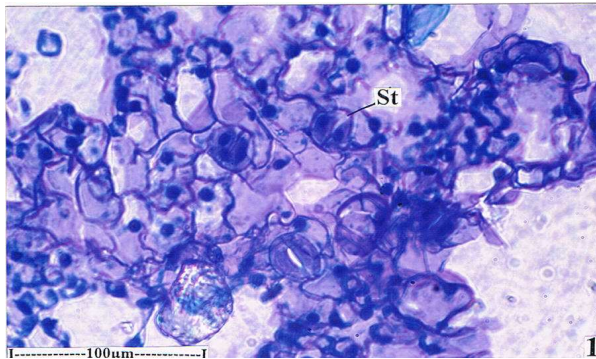


Fig. 9: Paradermal sections showing stomata
(St: Stomata)

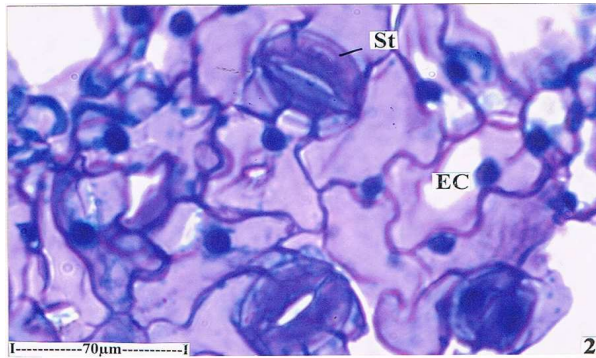


Fig. 10: Paradermal sections showing epidermal cells and stomata
(EC: Epidermal cells; St: Stomata)

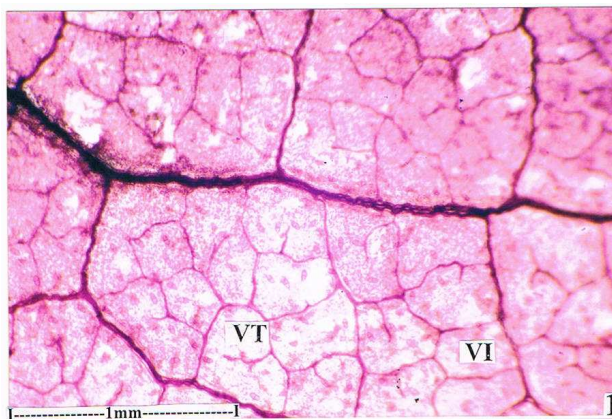


Fig. 11: Leaf cleared and stained with Saffranin showing venation pattern
(VI: Vein Islets; VT: Vein Termination)

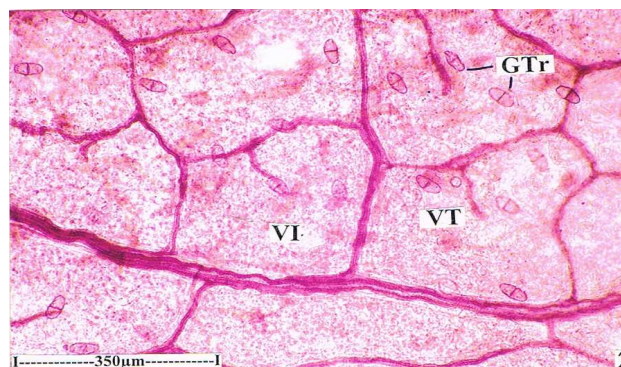


Fig. 12: Leaf showing venation pattern

(VI: Vein Islets; VT: Vein Termination; GTr: Glandular Trichome)

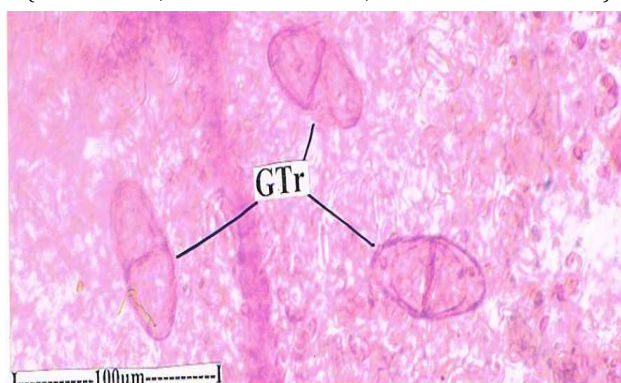


Fig. 13: Leaf showing epidermal glandular trichomes

(GTr: Glandular Trichome)

Glandular trichomes

Epidermal glandular trichomes (Fig.12 and 13) were common on the lower epidermis of the lamina. The glands were 20x50 μm in size, elliptical, two-celled and attached to the epidermis with a small stalk cell.

Phytochemical screening of *Artemisia Nilagirica* leaves

Phytochemical studies revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, tannins, phenol, terpenoids, saponins and essential oils in various extracts of *Artemisia Nilagirica* leaves. Summary of the screening results are listed in table 1.

Table 1: Phytochemical screening of *Artemisia Nilagirica* leaves

S. No.	Phytochemical and Test	PET	HEX	DEE	BEN	EDC	ETH	AQE
1	Test for alkaloids							
	(a) Dragendroff's test	A	P	P	P	P	P	P
	(b) Mayer's test	A	P	P	P	P	P	P
2	Test for amino acids							
	(a) Ninhydrin test	A	A	A	A	A	P	A
	(b) Cysteine test	A	A	A	A	A	P	A
3	Test for carbohydrates							
	(a) Barfoed's test	A	A	A	A	A	P	P
	(b) Fehling's test	A	A	A	A	A	P	P
	(c) Benedict's test	A	A	A	A	A	P	P
4	Test for flavonoids							
	(a) Ammonia test	P	P	P	P	P	P	P
	(b) Zinc HCl test	P	P	P	P	P	P	P
	(c) Aluminium tests	P	P	P	P	P	P	P
5	Test for glycosides							
	(a) Killer-Killani test	P	P	P	P	P	P	P
	(b) Baljet test	P	P	P	P	P	P	P
6	Test for Tannins & phenol							
	(a) Lead Acetate test	A	A	P	A	A	P	A
	(b) Ferric Chloride test	A	A	P	A	A	P	A
	(c) Ammonia test	A	A	P	A	A	P	A
7	Test for Terpenoids and steroids							
	(a) Salkowski test	P	P	P	P	P	P	P
	(b) Liebermann's test	P	P	P	P	P	P	P
8	Test for Saponins							
	(a) Froth test	A	A	A	A	A	P	A
9	Test for Lignin							
	(a) Saffranine test	A	A	A	A	A	A	A
10	Test for essential oil							
	(a) Sudan red test	A	P	A	A	A	P	A

PET: Petroleum ether; HEX: n-Hexane; DEE: Diethyl ether; BEN: Benzene; EDC: Ethylene dichloride; ETH: Ethanol; AQE: Aqueous; P: Present; A: Absent

CONCLUSION

Microscopical studies revealed *Artemisia Nilagirica* leaves consist of a thick midrib and thin lamina. The lamina was dorsiventral and exhibits dense reticulate venation which were thick, wide with distinct vein-islets. However, vein terminations were seen in most of the islets. The petioles were thick and prominent. The epidermal cells were wide, wavy, thick anticlinal walls and assume amoeboid outline. The stomata were anomocytic and have large guard cells with wide slit like aperture. Lower epidermis of lamina consists of glandular trichomes which were elliptical, two-celled and attached to the epidermis with a small stalk cell. Phytochemical studies of *Artemisia Nilagirica* leaves revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, tannins, phenol, terpenoids, steroids, saponins and essential oils in various extracts. These pharmacognostic and phytochemical characterization data would be helpful in authentication of raw material or crude drug of *Artemisia Nilagirica* leaves found in Nilgiris district of Tamil Nadu.

ACKNOWLEDGEMENTS

Authors are thankful to the Head of Pharmacognosy Department, Periyar College of Pharmaceutical Sciences, Trichy and Head of Pharmacy Department, Annamalai University, Chidambaram for providing all necessary facilities to carry out the work.

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