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RESEARCH ARTICLE

Pharmacognostical and Phytochemical Investigation of *Artemisia nilagirica* (Clarke)

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Abstract:
Artemisia nilagirica (Clarke) is an aromatic, small shrub belonging to the family Asteraceae. Leaves and flowering top of this plant reported to have anti-leishmanial activity and also use in traditional medicine as an antimalarial, nerve tonic and anticancer agent. Microscopic and physicochemical analysis of powdered sample of leaf of *Artemisia nilagirica* (Clarke) was studied for its morphological, microscopic, organoleptic characters and various other WHO recommended methods for standardization. Phytochemical study revealed the presence of various constituents such as alkaloids, amino acids, carbohydrates, flavonoids, glycosides, terpenoids, steroids, saponins, essential oils, tannins and phenol in various extracts. The data obtained through these pharmacognostic and phytochemical characterization would be helpful in authentication of raw material or crude drug of *Artemisia nilagirica* (clarke) leaves found in nilgiris district of Tamil Nadu.

Keywords: *Artemisia nilagirica* (clarke), Microscopy, Phytochemical analysis.

Introduction

Artemisia Nilagirica is popularly known as 'Nagdona' in Hindi. 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* (clarke) 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.1and 2).

Artemisia nilagirica (clarke) is used in traditional medicine for the treatment of various ailments such as poor appetite, indigestion, constipation, parasitic infection, cramp, cold, fever, gout, nervousness, leprosy, pruritus, malaria, fungal infection, leishmaniasis and cancer. This herb has also been used as antiseptic, antioxidant, anthelmintic 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* (clarke).[1-4]

Fig.1: Whole plant of *Artemisia nilagirica* (clarke)

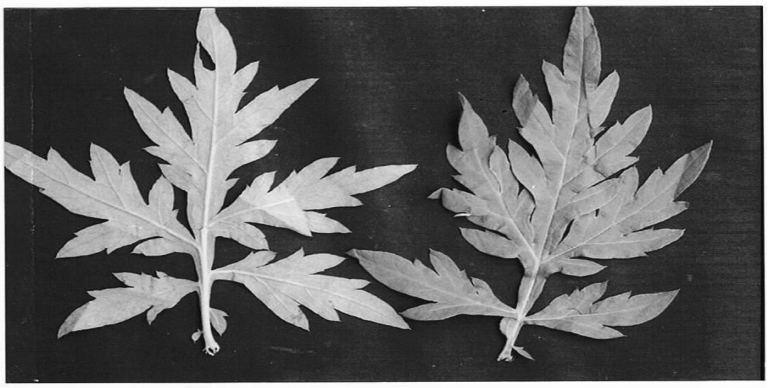

Fig. 2: Leaf of the *Artemisia nilagirica* (clarke)

Materials and methods

Collection and of authentication plant:

The plant was collected from Ooty district, Tamil Nadu, India in December 2011 and identified by Dr. N. Selvaraj Professor and head of Tamil Nadu Agriculture University, Horticulture Research Station Ooty, Tamilnadu India. The plant specimens were deposited in the herbarium Department of Pharmacognosy Mahakal Institute of Pharmaceutical Studies, Ujjain, Madhya Pradesh, Voucher no. MIPS/A/36/2011.

Reagents and chemicals

All chemicals and solvents used in this project were procured from Merck (Germany), SD Fine chemical (India), Loba, Research Lab and Ranchem (India).

Morphology

Morphology of the leaves was determined by placing them over the stage of a simple microscope and observing through a 6 X lens.

Organoleptic and Microscopic analysis

Healthy normal leaves of *Artemisia nilagirica* (Clarke) were cleaned using deionized water, dried in shade and crushed to yield coarse powder. The powder was stored in an airtight amber colored bottle throughout the study; it's 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+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. The powder was sensed for its color, odor, texture and taste by placing in a Petri dish. The color was reported by placing the powder against a white background and observing in day light. The odor and taste was evaluated by sensing a dry powder as well as placing a pinch of powder in warm water. Sections (10-12 µm 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.[5-13]

Phytochemical analysis

50 gm of the powdered *Artemisia nilagirica* (Clarke) leaves were extracted using different solvents such as petroleum ether, n-hexane, diethyl ether, benzene, ethylene dichloride, chloroform and ethanol successively by soxhlet apparatus. The extracted material was used for the phytochemical analysis. 1 gm of various extracts of *Artemisia nilagirica* (Clarke) 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 chemical analysis.[14-23]

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 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.

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.

Test for 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 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 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.

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 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 minute and mounted in glycerine. Appearance of red coloured oil globule under microscope indicates the presence essential oil.

Results and Discussion

Morphology of *Artemisia nilagirica* (clarke) leaves

The organoleptic evaluation have shown that the leaves are 5-20 cm long, pinnate, smooth, dark green on upper surface and pale green with small white hairs on the bottom. The texture is glabrous and pubescent. Table 1 summarises the morphological characters of *Artemisia nilagirica* (clarke)**.**

Microscopical Characteristics of *Artemisia nilagirica* (clarke)

Artemisia nilagirica (clarke) leaves consists of a thick midrib and thin lamina. 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 squarish 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.

C.S. of the Leaf:

In cross-sectional view, the leaf appears possessing prominent and thick midrib, with short wide adaxial cone and wide and thick abaxial part. The midrib is 700 µm thick and 600 µm wide. The adaxial cone is 100 µm in height and 150 µm wide. It consists of a prominent epidermal layer of squarish cells and two or three rows of collenchyma cells. The abaxial part also has thin, but distinct epidermal layer of cells and parenchymatous ground tissue; the cells are circular, thin walled and compact. The vascular strand of the midrib is single, large and top-shaped. It is 300 µm thick and 250 µm wide. It consists of about 10 thin parallel rows of xylem elements and fairly wide abaxial arc of phloem elements. The phloem arc is capped by narrow band of thick walled cells (Fig.3).

Lamina:

The lamina is 130 µm thick. It is dorsiventral and hypostomatic. The adaxial epidermis comprises dilated, elliptical or squarish cells with thick walls. The cells are 20 µm thick. The abaxial epidermis is thin and cylindrical and possesses stomata. The mesophyll tissue is differentiated into adaxial band of thick, light, less compact cells and abaxial three or four layers of spherical or lobed parenchyma cells with wide airchambers. Prominent lateral veins are seen placed in median part of the mesophyll. The lateral vein is circular with a circle of dialated hyaline bundle sheath parenchyma enclosing a small group of about four xylem elements and a cluster of phloem elements (Fig.4).

Leaf Margin:

The marginal part of the lamina is vertically bent down; it is thinner than the midpart and end is semi circular. The mesophyll tissue is less distinct into palisade and spongy parenchyma. The epidermal layers are as in the middle part of the lamina (Fig.5).

T.S. of Petiole:

The petiole is wide, thick and top-shaped with long lateral wings (Fig.6). It is 1.65 mm thick and 1.7 mm wide. The midrib consists of a thin continuous epidermis comprising small, less prominent rectangular cells. In the adaxial part, the epidermis is followed by four or five layers of angular collenchyma. Remaining part of the midrib has homogenous, circular compact parenchyma cells.

The vascular system of the midrib is multistranded. There is a large, bowl shaped median bundle and smaller, top shaped, two or three lateral bundles (Fig.7). The median bundle is 600 µm wide and 550 µm thick. It consists of fairly long, parallel lines of angular wide xylem elements and broad arc of phloem. There is a thick semicircular cap of thick walled cells on the phloem end (Fig. 8). The lateral bundles are also collateral with parallel lines of xylem and thick cap of phloem and sclerenchyma elements.

T.S. of Stem:

The stem is circular with shallow ridges and furrows. It is 1.8 mm thick. It consists of a thin continuous layer of epidermis. Inner to the epidermis, especially along the ridges, there are four or five layers of collenchyma. The pith portion and inner cortex have thin walled parenchymatous tissue (Fig. 9).

There are about 17 discrete vascular bundles separated from each other by narrow medullary rays. The vascular bundles are arranged in a ring around central pith (Fig.10). The vascular bundles are top-shaped and thick. They are collateral with inner part of parallel lines or scattered xylem elements and outer thin band of phloem elements. On the outer end of the vascular bundle occurs thick semicircular sclerenchymatous cap. On the inner side also is seen small mass sclerenchyma cap (Fig.11).

Phytochemical screening of *Artemisia nilagirica* (clarke).

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* (clarke). Summary of the screening results are listed in table 2.

Conclusion

The present study was carried out with a vision to setup standards that could be beneficial for detecting the authenticity of this vital medicinal plant. Numerical standards reported in this work could be useful for the compilation of a suitable monograph of *Artemisia*

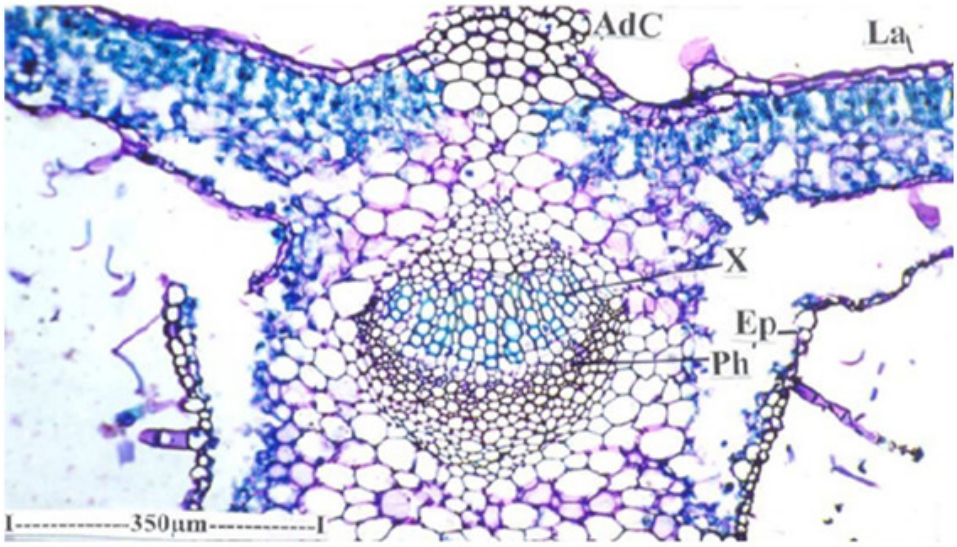

Fig. 3: C.S. of the Leaf

AdC - Adaxial Cone; La – Lamina; X – Xylem; Ep – Epidermis; Ph - Phloem

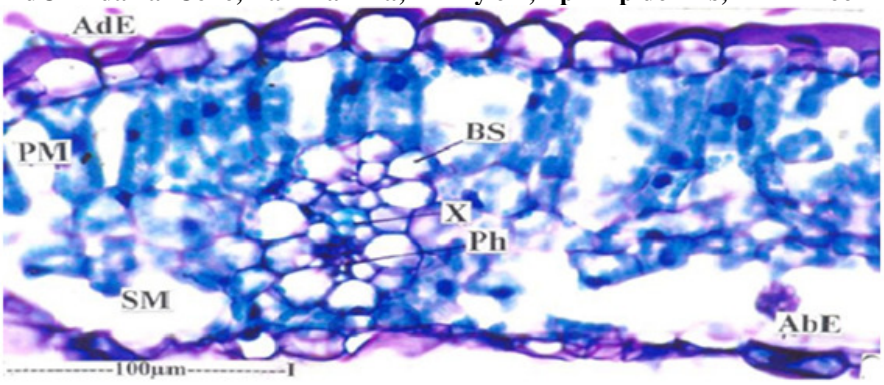

Fig. 4: T.S. of Lamina with lateral vein

AdE – Adaxial Epidermis; AbE - Abaxial Epidermis; BS –Bundle Sheath; Ep – Epidermis; La – Lamina; PM – Palisade Mesophyll; Ph – Phloem; X – Xylem

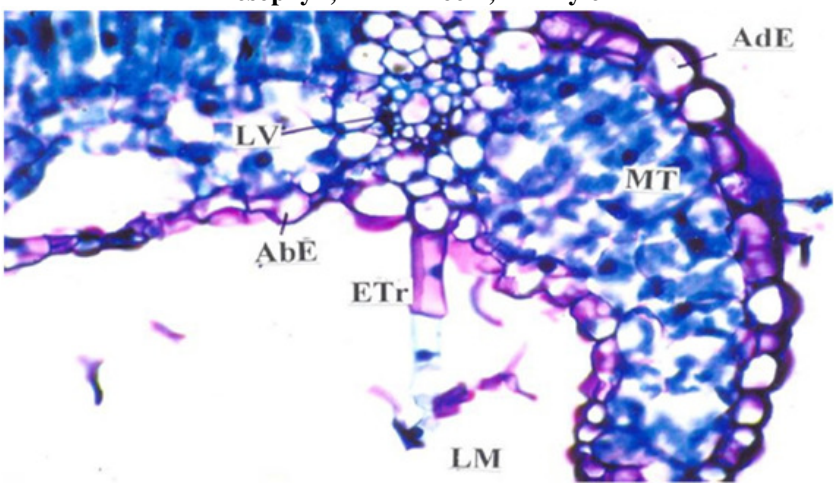

Fig. 5: TS of Leaf margin

AdE - Adaxial Epidermis; AbE - Abaxial Epidermis; ETr - Epidermal Trichome; LM - Leaf Margin

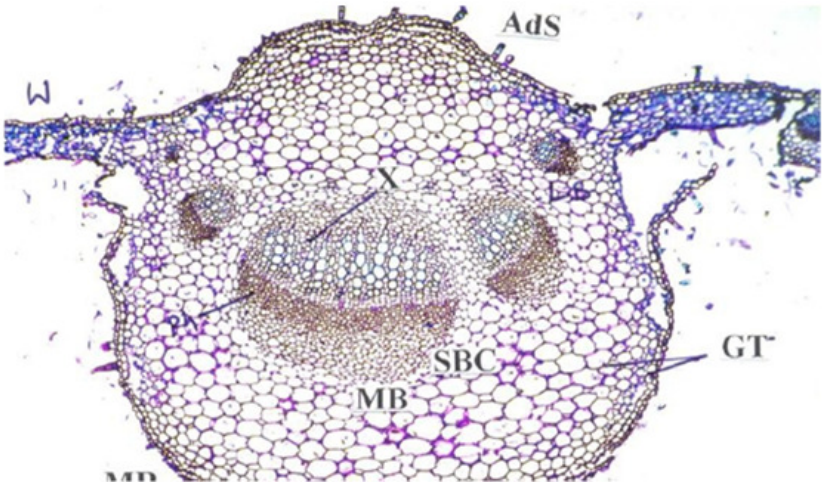

Fig. 6: TS of Midrib
AdS – Adaxial Side; MR – Midrib; MB – Median Bundle; SBC – Sclerenchyma Bundle ; W – Wing; GT – Ground Tissue

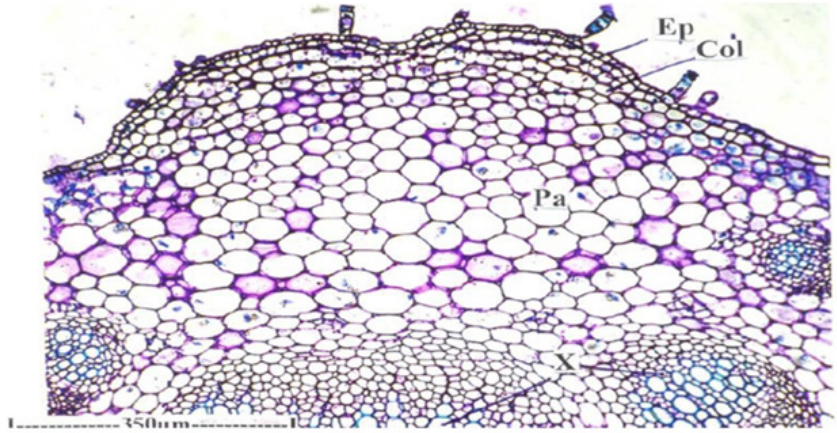

Fig. 7: TS of Midrib - Upper portion
Ep - Epidermis; Col - Collenchyma; Pa - Parenchyma; X – Xylem

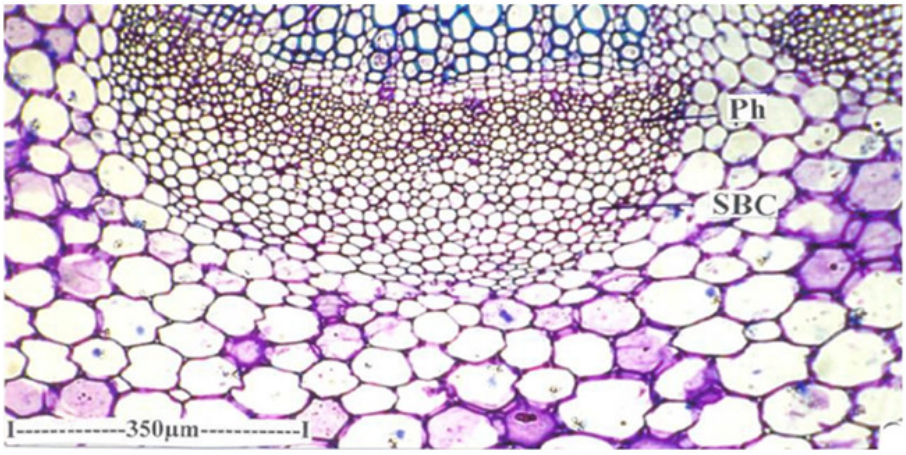

Fig. 8: TS of Midrib - Lower portion
SBC - Sclerenchyma Bundle; Ph - Phloem

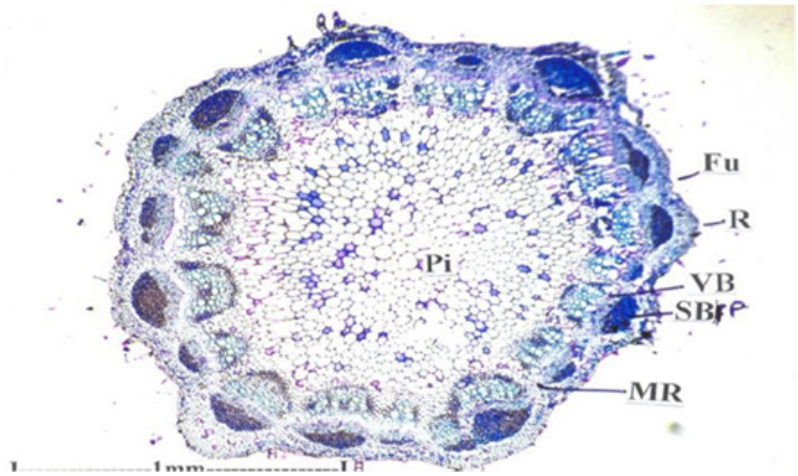

Fig. 9: TS of Stem: Ground Plant
Pi - Pith; SBC - Sclerenchyma Bundle Cap; Fu - Furrow; MR – Midrib

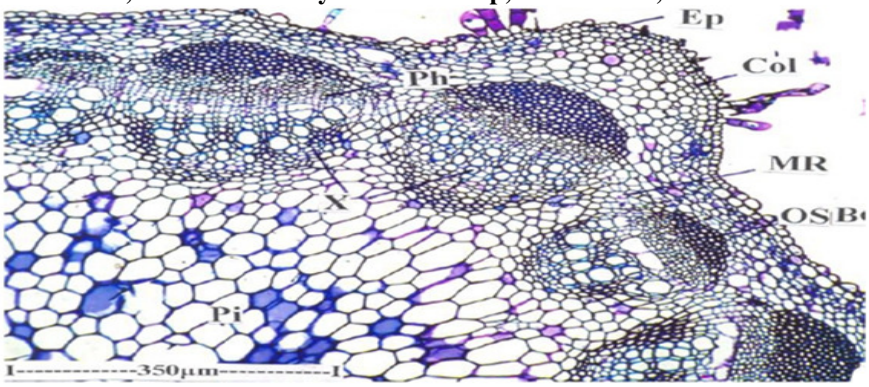

Fig. 10: A sector enlarged
Pi - Pith; SBC - Sclerenchyma Bundle Cap; Col - Collenchyma; MR - Medullary Ray; OSBC - Outer Sclerenchyma Bundle Cap; Ph - Phloem

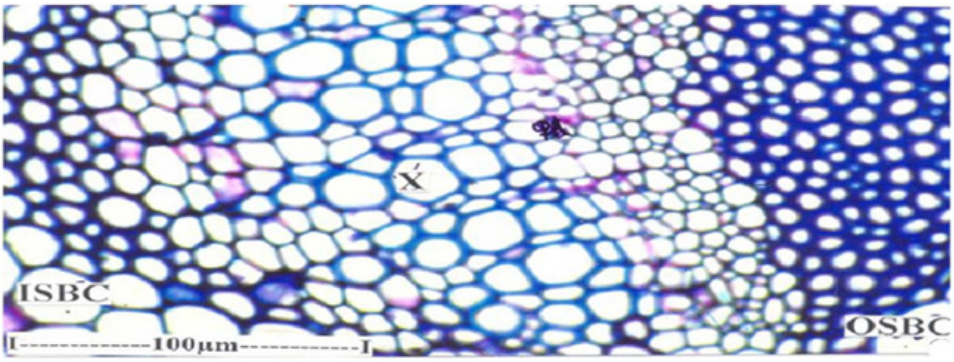

Fig. 11: A portion of the vascular bundle enlarged.
OSBC - Outer Sclerenchyma Bundle Cap; X - Xylem); OSBC - Outer Sclerenchyma Bundle Cap

Table 1 Morphological characteristic of *Artemisia nilagirica* (clarke) leaves

S.No.	Parameters	Observations
1	Color	Dark Green
2	Odour	Aromatic and pleasant
3	Taste	Bitter
4	Shape	Lanceolate
5	Taxture	Upper surface is glabrous and pubescent
6	Flower	In small globose heads
7	Fruits	Achene

Table 2: Phytochemical screening of *Artemisia nilagirica* (clarke) leaves

S. No.	Phytochemical tests	PET	HEX	DEE	BEN	EDC	CHF	ETH
1	**Test for alkaloids**							
	Dragondroff's	A	P	P	P	P	P	P
	Mayer's	A	P	P	P	P	P	P
2	**Test for glycosides**							
	Killer-killani	P	P	P	P	P	P	P
	Baljet test	P	P	P	P	P	P	P
3	**Test for tannin and phenol**							
	Lead acitate test	A	A	P	A	A	A	P
	Ferric chloride test	A	A	P	A	A	A	P
4	**Test for amino acids**							
	Ninehydrine test	A	A	A	A	A	P	P
5	**Test for carbohydrates**							
	Fehling's test	A	A	A	A	A	A	P
	Benedict's test	A	A	A	A	A	A	P
6	**Test for terpenoid and steroids**							
	Salkowaski test	P	P	P	P	P	P	P
	Liembermann's	P	P	P	P	P	P	P
7	**Test for flavonoids**							
	Ammonia test	P	P	P	P	P	P	P
	Alluminium test	P	P	P	P	P	P	P
8	**Test for saponine**							
	Froth test	A	A	A	A	A	A	P
9	**Test for lignin**							
	Saffranine test	A	A	A	A	A	A	A
10	**Test for essential oils**							
	Sudan red test	A	P	A	A	A	A	P

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

nilagirica (Clarke) leaves. Microscopical studies revealed *Artemisia nilagirica* (clarke) 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. Lower epidermis of lamina consists of trichomes. Phytochemical studies of *Artemisia nilagirica (clarke)* 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* (clarke) leaves.

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