

Morphology, Anatomy And Phytochemical Tests Of *Azadirachta Indica* A. Juss. Leaves

Phyo Moh Moh Zin¹, Khin Cho Cho Oo²

Abstract

The medicinal plant *Azadirachta indica* A. Juss belonging to the family Meliaceae. This plant well known in Myanmar as tamar. The leaves of *Azadirachta indica* A. Juss were collected from Thanlyin Township, Yangon Region. The collected plants were classified and identified by using literatures for morphological characters. In morphological study, the plants are large evergreen tree, leaves are imparipinnate compound, leaflets 8-6 opposite pairs. Inflorescence are axillary raceme, panicle. In histological study, the upper surface of stomata and trichome are absent. The lower surface of stomata are anomocytic type and unicellular uniseriate trichomes are present. In the diagnostic characters of the odour of leaves is aromatic. In the preliminary phytochemical tests, the present of alkaloids, α - amino acids, carbohydrate, reducing sugar, glycoside, phenolic compound, saponin, tannin, flavonoid, steroid and terpenoid but the absent of cyanogenic glycoside and starch.

Keywords: *Azadirachta indica*, anomocytic stomata, phytochemical

INTRODUCTION

Medicinal plants are rich source of novel drugs that forms the ingredient in traditional systems of medicines, modern medicines, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs. WHO pointed out that more than 80% of world's population depends on plants to meet their primary health care needs.

People have become favoured and accepted the safe and effective role of medicinal plants and traditional medicine knowledge and scientific reports. Meanwhile the exploration of the knowledge of plants used in traditional medicine is increased to disseminate as an important research area (Medicinal Plants of Myanmar, 2007).

An indigenous plant, *Azadirachta indica* A. Juss. belongs to the family Meliaceae. *Azadirachta indica* A. Juss., is known as "tamar" in Myanmar. The Meliaceae family distributed throughout the tropics and warm temperate region has about 53 genera and 1350 species (Bhattacharyya *et al.*, 1998). Chenallier *et al.*, (1996) described that the leaves are traditionally steeped for malaria, peptic ulcers and intestinal worms and herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Medicinal uses of *Azadirachta indica* A. Juss. have been scientifically shown to possess a variety of pharmacological properties such as antiinflammatory, analgesic, immunostimulant, hypoglycemic, antipyretic and antiulcer properties (Tomar, 2011).

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are chemical compounds that occur naturally in the medicinal plants (phyto means "plant" in Greek), leaves, vegetables and roots that have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Wadood *et al.*, 2013).

The aim of the research work is to explore the Myanmar medicinal plants and to promote the Myanmar medicinal plants and to promote the Myanmar traditional

¹ Assistant Lecturer, Dr., Department of Botany, East Yangon University

² Professor, Dr., Department of Botany, Panlong University

reproductive part of the plant, in the histological and diagnostic characters of leaves, to perform the phytochemical tests of leaves of *Azadirachta indica* A. Juss.

MATERIALS AND METHODS

Morphological study of *Azadirachta indica* A. Juss.

Collection and identification

The plant specimens of *Azadirachta indica* A. Juss. were collected from Thanlyin Township, Yangon Region, during the flowering and fruiting periods from January to July, in the year 2017. For morphological study, the specimens were measured, described in detail and identified with the help of available literatures (Hooker, 1875; Backer, 1965; Dassanayake, 1995). The specimens such as habit, leaves, inflorescences and flowers were recorded with the photographs.

Histological study of *Azadirachta indica* A. Juss.

In histological study, *Azadirachta indica* A. Juss. was prepared by free hand sections and examined according to the methods of Esau (1965), Tomlinson (1969), Trease and Evans (1978), Pandey (1998) at the Department of Botany, East Yangon University.

Phytochemical tests of powdered leaves from *Azadirachta indica* A. Juss.

Preliminary phytochemical investigation on leaves from *Azadirachta indica* A. Juss. was carried out to examine the plant constituents. The powdered leaves of *Azadirachta indica* A. Juss. was tested qualitatively for the presence or absence of alkaloid, α - amino acid, carbohydrate, starch, reducing sugar, cyanogenic glycoside, glycoside, phenolic compound, saponin, tannin, flavonoid, steroid and terpenoid. According to the methods of Central Council for Research in Unani Medicine (1987) and Trease and Evans (2002), the investigation of phytochemical studies was applied.

RESULTS

Morphological characters of *Azadirachta indica* A. Juss.

Scientific name	-	<i>Azadirachta indica</i> A. Juss.
English name	-	Mardosa or Neem Tree
Myanmar name	-	Tamar or Tamar- Kha
Common name	-	Indian Lilic
Family name	-	Meliaceae

Azadirachta indica A. Juss. is a large evergreen tree, about 7.0-12.0 m (40-55 ft) in height. Stems with long spreading branches forming a broad round crown woody hard. Leaves are alternate, imparipinnate compound, leaflets 8- 6 opposite pairs, the lamina oblong with 4.0-7.0 cm long and 1.5-3.2 cm wide, the base oblique, the margin serrate, the tip acute, petioles terete, 3.2- 6.0 cm long, pubescent. Inflorescence axillary raceme, panicle, 6.0-20.0 cm long; peduncles straight, 4.0-10.0 cm long. Flowers white, fragrant, about 4.0 mm long and about 10.0 mm in diameter, pedicellate, complete, bisexual, regular, actinomorphic, pentamerous, fragrant, hypogynous. Sepals 5, aposepalous, pale green, about 1.0 mm long and 2.0 mm in diameter, imbricate. Petals 5, apopetalous, white, spatulate, about 5.0 mm long and about 2.0 mm in diameter, imbricate. Stamens 10, monadelphous forming a tube, the staminal tube white, linear, about 1.0 mm long and about 0.5 mm in diameter, anthers ditheous, basifixed, introrse, basifixed, longitudinal dehiscence, a disc present between stamens and ovary. Carpel (5), ovoid, about 1.0 mm long and about 2.0 mm in diameter, syncarpous, two ovules in each locules, superior, axile placentation, styles cylindrical, 1.0-1.5 mm long, stigma capitate, about 0.5 mm long and about 0.3 mm in diameter. Fruit drupaceous, pale green, glabrous, oblong, about 1.4 cm long and 1.5 cm wide. Flowering and fruiting time from January to July, in the year 2017.

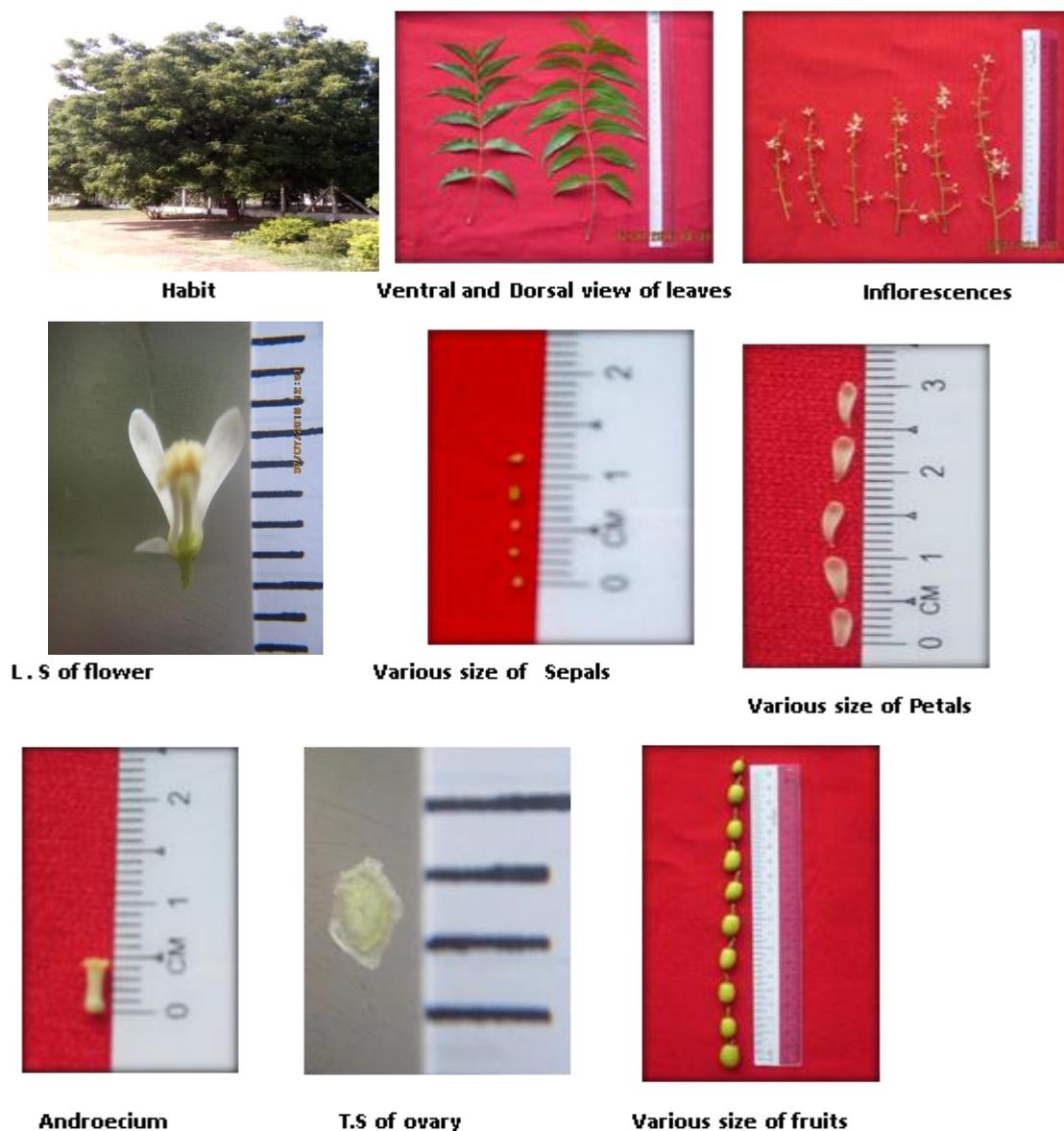


Figure 1. Morphological characters of *Azadirachta indica* A. Juss. leaves
Microscopical characters of *Azadirachta indica* A. Juss. leaves
Lamina

In surface view of lamina, the epidermal cells of upper surface are polygonal shaped and anticlinal walls are straight. The epidermal cells of lower surface are polygonal shaped and anticlinal walls are slightly wavy. Anomocytic stomata type are present.

In transverse section, the upper epidermis of cuticle layer is thicker than the lower surface. The upper and lower epidermal cells are barrel-shaped. Unicellular uniseriate trichomes are present on upper surface only. The mesophyll cells consist of one layer of palisade parenchyma and four to five layers of spongy mesophyll cells.

Vascular bundles are embedded in mesophyll tissues. Bundles are collateral and closed type. Xylem lies towards the upper epidermis and consists of thickened vessels, tracheids, fibres and xylem parenchyma. Phloem present toward the lower epidermis and consist of sieve tubes, companion cells, phloem fibres and phloem parenchyma cells.

Midrib

In surface view of midrib, the epidermal cells of both surfaces are parenchymatous, thin-walled, polygonal shaped and anticlinal walls are straight. Unicellular uniseriate trichomes are present on both surfaces.

In transverse section, the epidermal cells are one-layered which are rounded to oval in shape, parenchymatous cells. Below the epidermis, angular collenchyma and parenchyma layers are present. They composed of four or five layered of angular collenchymatous cells and three to five layers of parenchymatous cells which are located above the vascular bundles. Patches of sclerenchymatous cells are present.

The vascular bundles are crescent shaped. The vascular bundle of midrib is collateral and closed type. The xylem cells are endrarch, hexagonal and are arranged in radial rows, composed of vessels tracheids, fibres and xylem parenchymatous cell. The phloem cells are small and composed of sieve tubes, companion cells, phloem, fibres and phloems parenchymatous cells.

Petioles

In surface view, the epidermal cells of both surfaces are thin-walled parenchymatous cells, polygonal shaped and anticlinal walls are straight. Unicellular uniseriate trichomes are present on both surfaces.

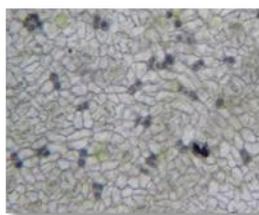
In transverse section, petioles are oval-shaped in outline and winged petiole are present.

Single layer of epidermal cells are barrel-shaped, cuticle smooth in both surfaces. Below the epidermal cells, three to five layers of angular collenchymatous cells, two to three layers of parenchymatous cells are present.

Vascular bundles are collateral and closed type. Xylem are endarch. Xylem lies on the upper epidermis and consists of vessels, fibres and xylem parenchyma. Phloem lies on the lower surface and made up of sieve tubes, companion cells and phloem parenchyma.



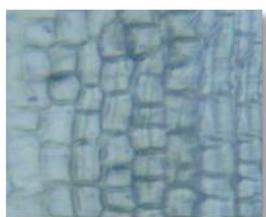
Upper surface view of lamina showing epidermal cells (x400)



Lower surface view of lamina showing epidermal cells with stomata (x100)



T.S of lamina showing epidermis and mesophyll cells (x100)



Surface view of midrib showing epidermal cells (x400)



Unicellular uniseriate trichome of midrib (x100)



T. S of midrib showing parenchymatous cells and vascular bundles (x100)

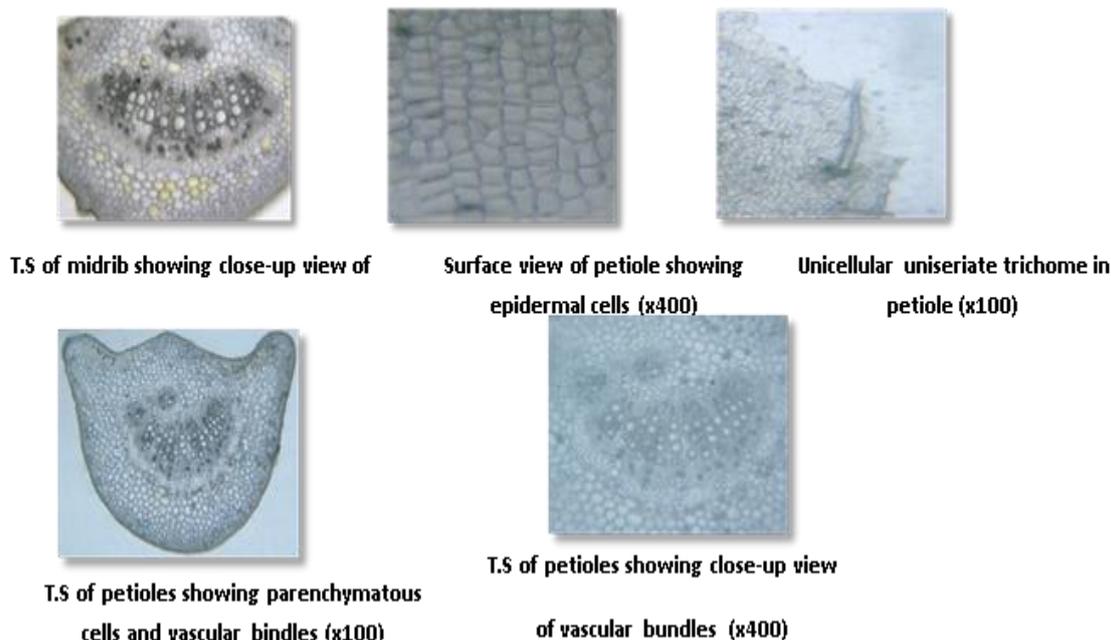


Figure 2. Microscopical characters of *Azadirachta indica* A. Juss. leaves

Diagnostic characters of powdered leaves of *Azadirachta indica* A. Juss.

In powdered sample of leaves *Azadirachta indica* A. Juss. are pieces of epidermal cells with anomocytic type of stomata, unicellular uniseriate trichome, fibres, pitted vessels, spiral vessel.

Table .1. Sensory characters of powdered leaves of *Azadirachta indica* A. Juss.

Sample (sensory characters)	Leaves
Colour	Green
Odour	Aromatic
Taste	Slightly Bitter
Texture	Fibrous

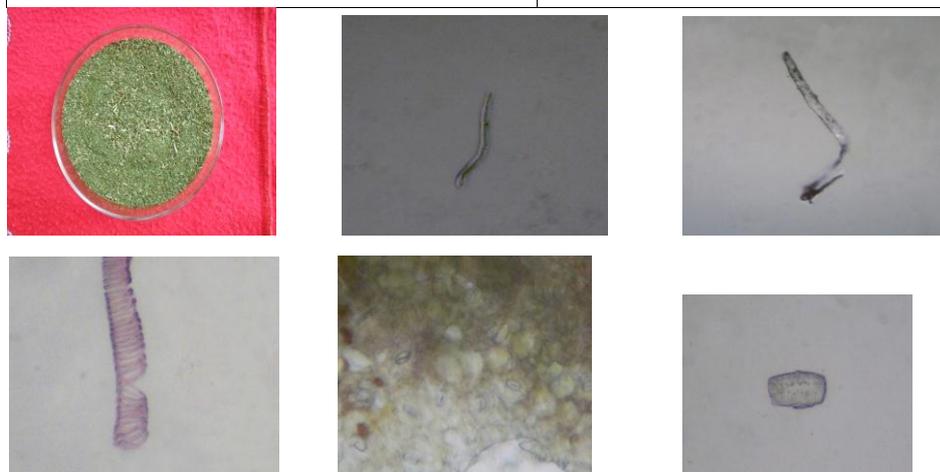


Figure 3. Diagnostic characters of *Azadirachta indica* A. Juss. Leaves

Phytochemical analysis of powdered leaves from *Azadirachta indica* A. Juss.

Phytochemical analysis of powdered leaves from *Azadirachta indica* A. Juss. had been carried out to know the presence or absence of alkaloid, flavonoid, glycoside, phenolic compound, tannin, reducing sugar, steroid, α - amino acid,

carbohydrates, saponin, cyanogenic compound, terpenoids and starch. Results were described in Table 2.

Table. 2. Preliminary phytochemical test of powdered leaves from *Azadirachta indica* A. Juss. Juss.

No	Test	Extrac t	Test Reagents	Observation	Result
1	Alkaloid	1% HCL	(1)Mayer's Reagent (2)wagner's Regent (3)Dragendroff's reagent	White ppt Brown ppt Orange ppt	+ + +
2	α -amino acid	H ₂ O	Ninhydrin solution	Purple color	+
3	Carbohydrate	H ₂ O	10% α -naphthol+conc-H ₂ SO ₄	Red ring	+
4	Starch	H ₂ O	I ₂ KI solution	Blue color	-
5	Reducing sugar	H ₂ O	Benedict's solution	Brick red ppts	+
6	Cyanogenic glycoside	H ₂ O	(1)conc-H ₂ SO ₄ acid (2) Sodium picrate paper	Yellow color	-
7	Glycoside	H ₂ O	10% lead acetate solution	White ppts	+
8	Phenolic compound	H ₂ O	Ferric chloride	Deep blue color	+
9	Saponin	H ₂ O	Distilled water	Frothing	+
10	Tannin	H ₂ O	Ferric chloride	Deep blue color	+
11	Flavonoid	EtOH	(1)Mg turning (2)Conc HCL acid	Pink color	+
12	Steroid	P.E	Acetic anhydride+conc-H ₂ SO ₄	Blue green color	+
13	Terpenoid	P.E	Acetic anhydride+conc-H ₂ SO ₄	Deep pink color	+

(+) = present, (-) = absence, ppt = precipitate, conc: = concentrated

DISCUSSION AND CONCLUSION

In the present research, the morphological characters on the vegetative and reproductive parts of the plant and histological study of leaves have been undertaken. The leaves are large evergreen tree, alternate, imparipinnate compound, the lamina oblong, the base oblique, the margin serrate and the tip acute. Inflorescences are axillary raceme, panicle. Flowers are white, pedicellate, complete, bisexual, regular, actinomorphic, pentamerous, fragrant and hypogynous. Androecium are monadelphous forming a tube, the staminal tube white, anther ditheous, introrse, basifixed and longitudinal dehiscence. These characters were in agreement with those given by Hooker (1875), Backer (1965), Dassanayake (1995).

In histological study, anticlinal walls of upper surface of lamina are straight and lower surface of lamina are slightly wavy. Anomocytic stomata type are present on lower surface. In transverse section of the upper epidermis of cuticle layers thicker than the lower surface. Unicellular uniseriate trichomes are present on upper surface only. These characters are similar to those mentioned by Esau (1965), Wallis (1967) and Evert (2006).

In transverse section of midrib are convex in upper region and concave in lower region, three to five layers of parenchymatous cells which located above the vascular bundles are closed collateral type. In transverse section of petiole are oval-shaped in outline and three to five layers of angular collenchymatous cells, closed collateral type. These characters were agreement with the findings of Esau (1965),

Tomar (2011) and Evert (2006). In powdered sample of leaves of *Azadirachta indica* A. Juss. are unicellular uniseriate trichome, fibres, spiral vessel, pieces of epidermal cells with anomocytic type stomata and pitted vessel were found. These characters are similar to those mentioned by Chenallier (1996). According to the above data, it is proved that the both morphological and histological characters of the specimen studied are useful for identification standardization of drugs.

In this study, phytochemical tests of *Azadirachta indica* A. Juss. leaves showed the presence of alkaloid, α - amino acids, carbohydrate, flavonoid, glycoside, saponin, tannin, reducing sugar, phenolic compound, steroid and terpenoid were detected. However, cyanogenic glycoside and starch were not found of leaves from *Azadirachta indica* A. Juss. These characters were agreement with those given by Itelima *et al.*, (2016). For further study, the other pharmacological effects of *Azadirachta indica* A. Juss. such as hypoglycemic activity, antipyretic activity, antioxidant activity should be carried out

ACKNOWLEDGEMENTS

I would like to acknowledge by sincere gratitude to Rector Dr. Kyaw Kyaw Khaung, East Yangon University, for his permission to present this research. I must especially express my deep gratitude to Dr. Ni lar Aung, Pro-rector, East Yangon University for her giving permission and suggestions. I would like to thanks Professor & Head Dr. San Khine, Professor Dr. Naw Thida Htoo, Department of Botany, East Yangon University, for providing the departmental facilities and their invaluable suggestions. I would like to express my deepest gratitude to my supervisor Dr. Khin Cho Cho Oo, Professor, Department of Botany, Panlong University for her overall supervision, in valuable opinions and collaboration in every aspect of my research.

REFERENCES

- Backer, C. A., Wolters., & Neordhaff, N. V., 1965. **Flora of Java**, Vol. III, Groninger, the Netherland.
- Bhattacharyya, B. B. M., & Johri, 1998. **Flowering Plants**, Central Reference Library, India: University of Delhi.
- Chenallier, A., 1996. **Encyclopedia Medicinal Plants**. London: Dorling Kindersley.
- Dassanayake, M. D., 1995. **A revised handbook to the Flora of Ceylon**, Vol. IX, University of peradeniya, Washington, DC. Amerind Publishind Co.Rt.Ltd.
- Esau, k., 1965. **Plant Anatomy**. 2nd Ed, Wiley and Sons, Inc, Hoboken, New Jewrsey, U.S.A.
- Evert, R.F. 2006. **Esau's Plant Anatomy**. Published by John Wiley and Sons, Inc, Hoboken, New Jewrsey, U.S.A.
- Harborne, J. B., 1984. **Phytochemical Methods**. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd; London.
- Hooker, J. D., 1875. **The Flora of British India**, Vol. I, Reeve Co., Ltd, The Oast House, Broct, NR. Ashford, Kent. England.
- Hundley H. G and U Chit Ko Ko, 1987. **List of Trees, Shrubs, Herbs and Principle Climbers**, Third Revised and Enlarge Edition, SUPDT, GOVT Printing and STATY, Union of Burma, Rangoon.
- Itelima J.U, 2016. *Phytochemical screening and antimicrobial activity evaluation of aqueous and ethanolic extracts of the leaf of Azadirachta indica Juss. (neem) on some microorganisms*, Vol.3, Pg.1, Department of Plant Science and Technology, University of Jos, Nigeria.
- Metcalf, C. R., & Chalk, L., 1969. **Anatomy of the Dicotyledons**, Vol. I, London: Oxford University Press.
- Pandey, S.N and A. Chadha, 1998. **Plant Anatomy and Embryology**. Vikas Publishing House Pvt. Ltd., New Delhi.
- Tomar, L. B. B., 2011. *Review on neem (Azadirachta indica): Thousand Problems One Solution*. *International Research Journal of Pharmacy*, Vol.XII, India. Pg, 97-102.
- Trease G.E. and W. C. Evans. 1978, 2002. **Pharmacognosy**. 13th, 16th ed. Elsevier Limited. London.
- Wadood, A., M. Ghufuran, S.B. Jamal, M. Naeem and A. Khan. 2013. Phytochemical analysis of medicinal plants occurring in local area Mardan, Parkistan.
- Wallis, T.E. 1967. **Textbook of Pharmacognosy**, 5th ed. Gloucester Place London; J and A. Churchill Limited.
- Central Council for Research in Unani Medicine**. 1987. *Phytochemical Standards of Unani Formulations*, New Delhi.