Pharmacognostical, Preliminary Phytochemical Evaluation of *Azima Tetracantha Leaves*

Prof. Dhanapal Venkatachalam[1*], Samuel Thavamani B[2], Hyfa[3], Sampath Kumar[4], Muddukrishniah[5]

[1] *Professor & H.O.D, Department of Pharmacognosy, Sanjo College of Pharmaceutical Studies, Velappara, Palakkad, Kerala, India*

[2]*Department of Pharmacognosy, Sanjo College of Pharmaceutical Studies, Velappara, Palakkad, Kerala, India*

[3]*Department of Pharmaceutical Sciences, Calicut Medical College, Kozhikode, Kerala, India*

[4]*Department of Pharmaceutical Sciences, Coimbatore Medical College, Tamilnadu, India*

[5]*Department of Biotechnology, Anna University Campus, Trichy, India*

**Corresponding author*

Abstract:

Objective:

To study of detailed pharmacognostic profile and preliminary phytochemical investigation and antifungal evaluation of leaves of *Azima tetracanthalam* belonging to the Family, Salvadoraceae commonly known as needle bush which is often used traditionally in Ayurveda for cough, phthisis and asthma,rheumatism,.dropsy, dyspepsia, chronic diarrhea, tooth ache and jaundice.

Methods:

Leaf sample of *Azima tetracanthalam* was studied by its Macroscopical, Microscopical, Physicochemical, Phytochemical analysis of powder of the plant and other methods for standardization recommended by WHO and also antifungal evaluation.

Results:

Macroscopically, the leaves are simple, opposite, elliptical orbicular, mucronate apex, pinnate, decussately arranged, pale green in colour, characteristic odour, and no taste. Microscopically, the leaf was showed the presence of dorsiventral shape, Anisocytic stomata, Polygonal epidermis, abaxial phloem, palisade parenchyma, radial xylem elements, vertical mass of hyaline, compact parenchyma cells, prominent cuticle, crystal sheath, absence of trichomes and sclerenchyma. These were the diagnostic features noted from anatomical study. Powder microscopy of leaf revealed the presence of parenchyma cells, xylem fibres and epidermis with anisocytic stomata and crystal sheath. The investigations also included leaf surface data; quantitative leaf microscopy. Physiochemical parameters such as loss on drying, extractive values and ash values were also determined. Preliminary phytochemical screening showed the presence of carbohydrates, flavanoids, tannins, phenolic compounds, alkaloids and glycosides.

Conclusions:

The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications.

Keywords: ***Azima tetracanthalam*, Microscopy, Macroscopy, Phytochemical evaluation**

I. INTRODUCTION

Medicinal plants are still used in major parts of developing countries as traditional medicinal systems to cure many infectious diseases with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many parts of the world.[1] Herbal medicine is still the mainstay of about 75–80% of the world's population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances. The strategy of isolating the active principles from the medicinal plants and manufacturing a pharmaceutical preparation then became popular. Modern medicines and herbal medicines are complimentarily being used in areas for health care program in several developing countries including India. Of late, the interest in the plant products surfaces all over the world due to the belief that many herbal medicines are known to be free from side effects. It is the fact that the discovery of the new synthetic drug is time consuming & an expensive affair[2]. The utility of the synthetic drug is always accompanied with its single or multiple adverse effects and in some cases the curatives are not available. Herbs had been used by all cultures throughout history but,India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants[3].In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential[4]. In spite of the tremendous advances made in the modern medicine there are still a large number of ailments for which suitable drugs are yet to be found. Today, there is an urgent need to develop safer drugs for the treatment of dreadful diseases. Hence, there is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine[5].

Azima tetracantha (Family:Salvadoraceae) is known as' needle bush' in English,'yasanku' in Malayalam, 'mulsangu' in Tamil and 'kundalin' in Sanskrit. It is a perennial shrub growing up to 3m height in hot, dry river in scrub, particularly on alluvial or saline soil. It is naturally occurring as spiny, evergreen shrub with a tendency to scramble. Grows up to 2 meters in height. The juice of the leaves is said to relieve the cough phthisis and asthma rheumatism. The plant is considered as a powerful diuretic and is also used to treat rheumatism, dropsy, dyspepsia, chronic diarrhoea and as a stimulant tonic for women after confinement .Locally, the traditional healers from Tirunelveli district of Tamilnadu are using root bark, paste with butter milk of this plant as potent remedy for jaundice Decoction of leaves is used for effective remedy for dysentery and joint pain

II. MATERIALS AND METHODS

Collection and Authentication:

Azima tetracantha leaf was collected, from Palakkad, Kerala, India and authenticated by taxonomist and the authenticated plant specimen was deposited in the Department of Pharmacognosy, Sanjo college of pharmaceutical studies, Palakkad. Authentication specimen number is SCPS/P.COG/008/2018 the fresh leaves were kept for shade drying. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Pharmacognostic Standardization:

Organoleptic characters such as shape, size, colour, odour, taste of were determined. Microscopic studies was carried out by preparing thin hand section of leaf with Chloral hydrate solution, stained with Phloroglucinol-hydrochloric acid (1:1) and mounted in glycerine[6.] Histochemical studies and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the material.[7]

Physico-chemical Evaluations:

The parameters were done to evaluate the proceedings of total ash; water soluble ash; acid insoluble ash and sulphated ash were calculated as per Indian Pharmacopoeia.[8] Extracts of the powdered leaf was prepared with different solvents for the study of extractive value..

Preliminary Phytochemical Screening:

The methanol, petroleum ether, ethyl acetate, n-hexane and aqueous extract of. *Azima tetracantha* was subjected to tests for the presence or absence of the major class of compounds by standard methods.[9] Fluorescence analysis was also carried out for the powder and for extract as per standard procedure[10].

Extraction of Plant material

For preliminary phytochemical analysis, extract was prepared by weighing 1kg of the dried powdered aerial parts were subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, chloroform, methanol, ethanol and finally with aqueous. The extracts were filtered in each step using Whatman filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45 ^{o}C) and pressure. The presence or absence of the primary and secondary phytoconstituents was detected by usual prescribed methods[11]

Powdered drug reaction with different reagent:

Powder drug was treated with different reagent and was observed from naked eye.

III. RESULTS

Macroscopical characters:

Leaves are pale green coloured , the size was 1.5 to 5.5cm long 0.5 to 4.5cm in width and are arranged decussately opposite, elliptical in shape, with mucronate apex , the leaf base is pinnately veined with one pair of lateral vein. Orbicular in shape, entire margin, decussately arranged, characteristic odour and no taste.

Figure 1- Habit profile of leaf of *Azima tetracantha*

Figure 2-Macroscopy of the leaf- Ventral view

Figure 2-Macroscopy of the leaf- Dorsal view

Histological characters:

The detail and systemic Pharmacognostical evaluation would give valuable information for the future studies. Transverse section of the leaf shows dorsiventral nature of the leaf. Following are the important tissues in the midrib regions, lamina, and petiole.

Midrib:

The midrib was flat on the adaxial side and hemispherical on the abaxial side. The midrib was 400µm in vertical plane and 200 µm in horizontal plane. The epidermal layer of the midrib was thin, rectangular with prominent cuticle; the abaxial epidermis thinner and the cells are spindle shaped. The ground tissue on the adaxial part consists of a vertical mass of hyaline, compact parenchyma cells. The abaxial midrib had small, compact, thin walled parenchyma cells. The vascular bundle was single, top shaped and consists of a few parallel, short, radial multiples of vessels and an abaxial arc shaped phloem. There is no distinct bundle sheath; no sclerenchyma elements were seen in the vascular bundle.

Lamina:

The lamina was 230 µm thick. Both adaxial and abaxial sides were smooth and even; no trichrome were evident. The adaxial epidermis was slightly thicker than the abaxial epidermis; it consists of horizontally rectangular cells with distinct cuticle. Beneath the epidermal was a single layer of large, hyaline, rectangular hypodermal layer of cells. The abaxial epidermis consists of narrow, spindle shaped cells; this layer was stomatiferrous. The mesophyll tissue was differentiated into adaxial palisade zone, which was 80 µm in height and it consists of two layers of short, less compact thin walled cells. The lower zone was spongy mesophyll which had four or five layers of lobed, loosely arranged cells. The vascular bundles of the lateral veins were embedded in the median part of the mesophyll tissue.

Epidermal tissues:

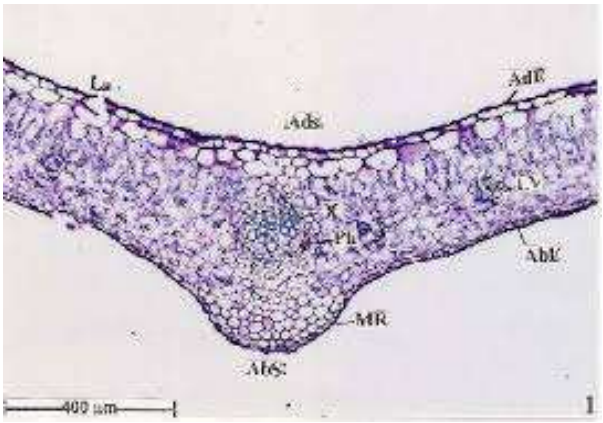
The epidermal tissue as seen in paradermal section. It consists of stomata and epidermal cells. The stomata ere tetracyclic with four subsidiary cells or anisocytic with four unequal subsidiary cells. The guard cells were uniformly elliptical with prominent nuclei. The epidermal cells were polygonal or more predominantly rectangular in surface view. They have prominent nuclei. The anticlinal walls of the epidermal cells are straight and fairly thick.

Petiole:

The basal and upper parts of the petiole differ in cross sectional outline; but the ground tissue and the vascular bundle remain similar. The basal and upper part of the petiole was circular in outline in measuring 1.15 µm in diameter. The surface was smooth and even. The petiole had thin, continuous epidermis made up of thick walled elliptical epidermal cells. The ground tissue had homogenous, parenchymatous, compact thin walled cells. The vascular strand was single, collateral and deeply arc shaped. It consists of closely arranged parallel, radial files of xylem elements and a thin continuous arc of phloem on the abaxial sides of the xylem band.

An arc of phloem occurs in a discontinuous row of sclerenchyma patches. The distal (upper) part of the petiole was 1mm in horizontal plane and 850µm in vertical plane. It was semi-circular in outline; the adaxial side was flat with short, thick lateral wings. The abaxial part was semi-circular and even. It consists of thin epidermis, compact, homogenous, thin walled parenchymatous ground tissue and single, collateral arc shaped vascular bundle, supported byabaxial small masses of sclerenchyma cell

Figure 3- T.S of leaf through midrib and lamina



La-Lamina,**Ads**, Adaxial side;

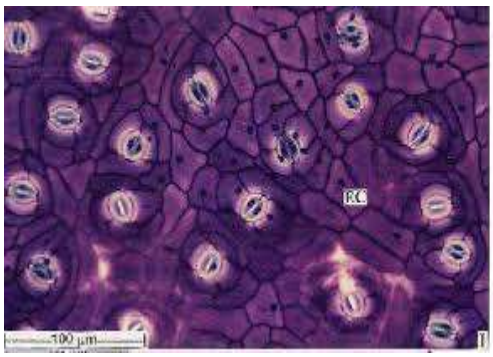
AdE, Adaxial epidermis;**X**,Xylem;

Ph, Phloem; **LV,** Lateral vein;

AbE,Abaxial epidermis; **MR**, Midrib;

AbS, Abaxial side.

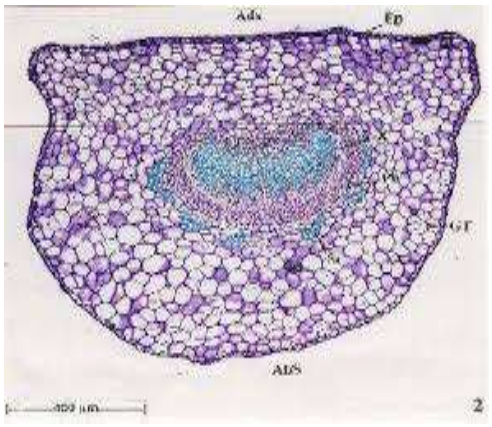
Figure 4- Stomata



EC, Epidermal cell; **SC**, Subsidiary cell

GC. Guard cell

Figure 5 T.S of petiole



Ads, Adaxial side; **Ep,** Epidermis; **AbS,** Abaxial side; **GT,** Ground tissue; **X**, Xylem; **Ph,** Phloem; **Sc,** Schlerenchyma.

Powder microscopy:

Powder characteristics revealed the presence of xylem,phloem, anisocytic stomata,crystal sheath, fibres,vessel elements,parenchyma and sclerenchyma.

Quantitative microscopy:

The quantitative microscopy such as vein- islet number, vein- terminal number, stomatal number and stomatal index were determined and the results were tabulated. (Table 1)

Table 1: Quantitative evaluation of the crude drug of leaf of *Azima tetracantha*

Standardisation parameters	values
Vein islet number	12/sqmm
Vein termination number	17/sqmm
Stomatal number lower epidermis	15.66
Stomatal index –Lower epidermis	19.5

Physiochemical parameters:

The powdered drug was evaluated for its physico-chemical parameters like total ash values, acid insoluble ash, water soluble ash and loss on drying, and the results were tabulated (Table 2). The ash contents showed the amount of inorganic matter present in the sample and the acid insoluble ash almost within 2.2 % which expresses low siliceous matter present in the sample.

Table 2. Physico chemical evaluation of *Azima tetracantha*

Standardization parameters	% W/W
Total Ash	7.45±0.03
Acid Insoluble Ash	2.20±0.02
Water Soluble Ash	6.98±0.023
Loss on Drying	9.17

Extractive values:

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug and the results were tabulated (Table 3).

Table 3 - Extractive values of leaf extracts of *Azima tetracantha* with different solvents

S.No.	Extracts	Extractability (%)
1.	Petroleum Ether Extract	2.9
2.	Benzene Extract	2.65
3.	Chloroform Extract	3.45
4.	Methanol Extract	1..2
5.	Ethanol Extract	1.197
5.	Aqueous Extract	1.593

Preliminary phytochemical analysis:

The powdered drug and various extracts such as petroleum ether extract, benzene extract, chloroform extract, methanolic extract, ethanol extract and aqueous extract were subjected to preliminary phytochemical screening of their presence or absence of the constituents and the results were tabulated (Table 4).

Table 4. Preliminary phytochemical tests for drug powder and various extracts of *Azima tetracantha*

Test	Drug powder	Petroleum ether	n-Hexane	Ethyl acetate	Ethanol	Aqueous
Sterols	-	+	-	-	-	+
Terpenoids	-	-	-	-	+	+
Carbohytrates	+	+	+	+	+	+
Flavanoids	+	+		+	+	+
Proteins	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Glycosides	-	-	+	-	-	+
Saponins	-	-	-	-	+	+
Tannins	+	-	-	-	+	+

Mucliage	-	-	-	-	-	-
Resins	-	-	-	-	+	-
Oil/Fats	-	-	-	-	-	-

Fluorescence analysis:

The powdered drug and they were treated with different solvents and the colour changes were observed under UV light in different wave length and the results were tabulated (Table 5)

Table 5- Fluorescence analysis of *A.tetracantha* powder

Solvents used	Day light	U.V light 254nm	365nm
1 N NaOH (Aqueous)	Orange	Yellowish green	Black
1 N NaOH (Alcoholic)	Light green	Green	Dark green
1N HCl	Pale yellow	Black	Black
50%H_2SO_4	Reddish brown	Dark brown	Black
50% HNO_3	Orange yellow	Green	Black
Picric acid	Yellow	Green	Black
Acetic acid	Brown	No visible colour	Black
CON HNO_3	Brown	Green	Black
$FeCl_3$	Orange	Green	Black
HNO_3+ NH_3	Reddish orange	Green	Black
Powder as such	Pale brownish yellow	Pale green	Brown

IV. DISCUSSION

Our study has focused on examining Pharmacognostic and Preliminary phytochemical study of. leaves of *Azima tetracantha*. Normalization of the macroscopic and microscopic characteristics of the *Azima tetracantha.* drug remains essential in other to identify and avoid falsification. Thus comparing the cross section of the leaf has a spinal cord parenchyma, a phloem, xylem and collenchymas. It is also observed that the adaxial epidermis is slightly thicker than the abaxial epidermis; it consists of horizontally rectangular cells with distinct cuticle. The lower zone is spongy mesophyll which has four or five layers of lobed. Organoleptic characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs [12].Thus leaves are pale green coloured , the size was 1.5 to 5.5cm long 0.5 to 4.5cm in width and are arranged decussately opposite, elliptical in shape, with mucronate apex , the leaf base is pinnately veined with one pair of lateral vein. Orbicular in shape, entire margin, decussately arranged, characteristic odour and no taste.

The micrograph performed on the powder has highlighted a number of characteristic elements namely: xylem, phloem, anisocytic stomata, crystal sheath, fibres,vessel elements, parenchyma and sclerenchyma. These diagnostic elements are consistent with botanical standards and WHO guidelines [13-14] .The study of physicochemical parameters such as moisture content and ash values are useful as it determines the physiological and nonphysiological state of ash, this will help to determine the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of 09.17 ± 0,1, which is below 10%. This result comply with the standards established by the International Pharmacopoeia, because this water content rate , prevent oxidation reactions , fermentation and give less chance to microbial growth and contamination in drugs[15]. Therefore, for proper conservation of drugs made from the leaves of. *Azima tetracantha.*, it would be desirable to use those whose water content is less than or equal to 10%. The determination of total ash gave us a rate of 7.45 ± 0, 03. This value indicates the level of minerals in drugs. Insoluble ash in hydrochloric acid gave a rate of 1.06 ± 0.02. Indeed, the ash insoluble in hydrochloric acid tells us about the contamination of the drug by siliceous elements[16]. This result is in agreement with Srikanth et al.[17] who found rate of 0.97% and 0.5% respectively. The maximum extractive value was found in Chloroform (3.45%) followed by Pet.ether (2.9%) Benzene (2.65%), Aqueous (1.593%),Methanol (1.2%), Ethanol (1.192%). All the extracts of the drug was subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed the presence of carbohydrates, flavanoids, tannins, phenolic compounds, alkaloids and glycosides. Preliminary phytochemical analysis indicated a high percentage of quercetine and flavonoids and this may be one of the reasons behind the anti diabatic and hepatoprotective activity of this plant. Though *A.tetracantha* is a weed, it is a highly reputed drug used in Ayurveda. Barring the anatomical details and preliminary phytochemical screening, rest of the pharmacognostical parameters, gives us the clue that it can be cashed economically as well to improve the standard of health in the developing countries.

V. CONCLUSION

WHO has emphasized the need to ensure quality control of the raw materials used for Ayurvedic medicines by using modern techniques and by applying suitable parameters and standards. In the present study various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, and preliminary phytochemical investigation were studied, which are being reported for the first time in this plant and could be helpful in authentication and preparation of a suitable monograph for the proper identification of *A.teteracantha* for the future.

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