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PHARMACOGNOSTIC PROFILING AND PHYTOCHEMICAL EVALUATION OF AZIMA TETRACANTHA LAM. (SALVADORACEAE)

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Abstract

Standardization in medical practice is a system which ensure that every packet of medicine that is sold maintains the quality and quantity of the requisite bioactive compound and will induce the desired therapeutic effect. Azima is an important medicinal shrub used both in traditional system of medicine as well as in Ayurveda. The present investigation is concerned with the pharmacogonostic standardization and phytochemical screening of a very common species of Azima, *Azima tetracantha*. A morphological study of the plant was conducted. The whole plant was shade dried and ground to fine powdered and subjected organoleptic, fluorescent and physicochemical analysis. The plant powder was subjected to Soxhlet extraction using methanol. Phytochemical screening (Qualitative, Quantitative) of the methanol extract of the plant sample was conducted according to standard biochemical procedures. Antioxidant activity of the plant extract was tested using ABTS assay. The organoleptic analysis revealed the characteristic color, taste, odor and nature of the powder of *Azima tetracantha*. In fluorescence analysis, on treatment with different solvents, color changes could be noticed in the plant powder. The results of the physicochemical analysis provide an important parameter in detecting adulteration or improper handling of drugs. Qualitative and quantitative analysis confirmed the presence of many important phytochemicals in the plant. Bioactivity study indicated the anti-oxidant potential of the plant comparable to that of the standard Trolox. Further work should be carried out to isolate, purity and characterize the active constituents responsible for the specific activity of the plant. Also, additional work is necessary to elucidate the possible mechanism of action of the extract.

Key words: , Azima, Pharmacognosy, Fluorescence, Physio chemical, DPPH

Introduction

The importance of plants to humans and just about all other life on Earth is fascinating. Plants fulfil the three basic needs of man such as food, clothing and shelter. They are also sources of fuel, building materials, craftwork material, dyes, transportation, rituals and medicine. Evidently, over the years, man has been consistently exploiting plants for his various needs. Medicinal plants which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the values of medicinal plants as potential sources of lead compounds in drug development. However, mindless and unplanned felling of trees, shrubs and herbs has resulted in the loss of existing biodiversity (Gayathri *et al*., 2012).

The candidate plant, in the present investigation, *Azima tetracantha* Lam. is a member of the family Salvadoraceae (Mustard tree family). It is used in traditional medicine practices in the remote villages of South Kerala. The plant is reported to have antispasmodic, diuretic, analgesic, wound- healing, anti-inflammatory, disinfectant, odontalgic, tonic, stimulant, anti-diabetic, anti-diarrhoeal, anti- arthritic, expectorant, anti-rheumatic, anti- catarrhal, antiperiodic and astringent properties. It is used to get relief from muscular rheumatism and in diarrhoea and is also given in dropsy, to relieve cough of phthisis and asthma; for ulcers and in small pox (Rama Rao, 1914).

Relevance of the present study

Traditional medicines still remain the main recourse for a large majority of people for treating health problems. Most of the plants used in herbal medicine practices, used by plant healers of remote villages and primitive aborigines have not yet been completely investigated for their phytochemical constituents and pharmacological activities. *Azima tetracantha* Lam.is one such plant used in the folklore herbal medicine practices in the villages of South Kerala. Almost all the studies on A. *tetracantha* were

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conducted in the recent past (later than 2000). Therefore, it appears that researchers became aware of the tremendous medicinal potential of *A. tetracantha* quite recently.Evaluation of the biopotential of *A. tetracantha,* and its phytoconstituents are scarcely reported from plant materials growing in the state of Kerala. *Azima tetracantha* is very prevalent in the adjacent state of Tamil Nadu and a few reports on the phytoconstituents and bioactivity have been reported from Tamil Nadu but not so from Kerala.

Materials and Methods

Plant Material

The plant selected for study, *Azima tetracantha* were collected from Chithara, Kollam. The specimen was authorized by Curator, Department of Botany, University of Kerala, Thiruvananthapuram.

I. Morphological Study

The morphology of the species *Azima tetracantha* was studied using taxonomic kit based on qualitative and quantitative characters. The observations were recorded.

II. Powder analysis

Fresh plant of *Azima tetracantha* is collected in polythene bag. The collected materials were washed under running tap water to remove adhered dirt. It was then rinsed with distilled water, blotted and dried in shade. The shade dried specimen was powdered in a mixer and sieved with fine mesh sieve. The powder was then used for the organoleptic study and solvent extraction.

Organoleptic study

Organoleptic (literally '[impression on the organs') refers to evaluation by means of the organs of sense and includes the macroscopic appearance of the plant material, its color, odour and taste, occasionally the sound to 'snap' of its facture and the 'feel' of the powder to the touch (Wozniak *et al*.,1997). The plant powder characteristics like the colour, odour, taste and nature were evaluated.

Fluorescence analysis

The crude drug powder was treated as such with eight different reagents solvents used were water, hydrochloric acid, sulphuric acid, sodium hydroxide, nitric acid, acetic acid, and methanol. Each solution was loaded on an activated thin gel layered slide and the fluorescence under normal light, short UV (100 nm) and long UV (320 nm) was observed (Chase and Pratt, 1949).

Physicochemical characterization

Different physcio-chemical parameters were determined according to the official methods and guidelines on quality control for medicinal plant materials.

1. Loss on drying (Indian Pharmacopoeia,1992)
2. Foaming index (WHO, 1992)
3. Swelling index (WHO,1992)
4. Foreign matter (Indian Pharmacopoenia,1996)
5. pH (Iqbal et al.,2010)

III. Phytochemical screening

A. Preparation and yield of extract (Indian Pharmacopoeia, 1996)

About 15 gm of the powdered plant material was subjected to extraction by Soxhlet apparatus using 100ml of methanol. The extract was concentrated under reduced pressure and preserved in refrigerator until further use. The percentage of the crude extract was determined using the following equation:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of the crude extract}}{\text{weight of the sample}} \times 100$$

B. Quantitative analysis (Harbone,1973).

1. Tannins
2. Saponins
3, Flavonoids
4. Alkaloids
5. Terpenoids
6. Phlobatannins
7. Glycosides
8. Simple phenolics
9. Coumarins
10. Quinones
11. Acids
12. Flavanols
13. Lignin
14. Steroids
15. Gums and mucilage

C. Quantitative analysis

1. Determination of Alkaloids
2. Determination of steroids (Okeke and Elekwa, 2003)
3. Determination of phenols (Spanos and Wrolstad, 1990

Bio-activity study—Anti-oxidant activity

1.Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging activity (Miller and Rice-Evans,1997)
Trolox standards were prepared of different concentrations. The ABTS substrate working solution was prepared by adding 25µl of 3% hydrogen peroxide solution to 10 ml of ABTS substrate solution. The assays were prepared in 96 wells plate. In the wells for the Trolox standard, 10µl of Trolox and 20µl of Myoglobin working solution were added. In the wells for the test samples, 10µl (20-80µg/ml concentrations) of plant extracts and 20µl of myoglobin working standard were added. Then solution was incubated for 5 minutes at room temperature. The stop solution (100µl) was added to each well. The end point absorbance was read at 405 nm using a plate reader

$$\text{Percentage of inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Result and Discussion
I. Morphological study
An account of the morphological characters of *Azima tetracantha* Is given below (Table 1).

II. Powder analysis
A. Organoleptic study
Colour – Light olive green
Smell – Pungent smell
Taste – Bitter
Texture – Smooth

B. Fluorescence analysis
The dry powder was subjected to fluorescence analysis with different reagents in normal light, short UV and long UV. The colour changes are summarised (Table2).

C. Phytochemical characterization
A total of five physio-chemical parameters were evaluated in *Azima tetracantha* (Table 3). The moisture content was reported to be in low amounts in the plant. Forming index was found to be more than 100 units and no considerable swelling was observed.

I. Phytochemical screening
A. Yield of the extract
The methanol extract was prepared by Soxhlet extraction. The yield of the methanol extract was 5.5%.

B. Quantitative analysis
A total of 15 phytochemical were qualitatively analysed in methanol extract of the plant. Most of the compounds were present in the extract. Coumarins, glycosides, quinones and mucilage were absent in the extract of the plant. The phytochemical screening tests are provided (Table 4 and Plate 2.)

C. Quantitative analysis
The quantitative estimation of three phytochemical was done in the methanol extract of *Azima tetracantha* (Table 5) by standard procedure. The amount of phenols was the highest and that of alkaloids was low.

D. Bioactivity study— Anti-oxidant activity

1.Azino–bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging activity (Miller and Rice-Evans,1997)
The scavenging activity of the extract was compared with that of Trolox standard and percentage inhibition was calculated (Graph 1). The percentage inhibition of the ABTS radical by the methanol extract increased with increase in concentration. In this assay, by Probit analysis the methanol extract of Azima had good ABTS radical scavenging activity with IC_{50} value of 39.45µg/ml. the IC_{50} value of the standard Trolox was 36 µg/ml. The results are provided (Table 6 and Plate 3).

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Table 1. Morphological characters of *Azima tetracantha*

Character	Description
Habit	Scrambling deciduous, shrub with axillary spines
Stem	Green herbaceous, branchlets branchlets terete or quadrangular. Young shoots pubescent, glabrous afterwards spines in each axial
Leaf	Greyish-green shining leaves with 2-3 pairs of prominent nerves from the base, spiny on tip. Leaves decussately opposite, simple and entire
Root	Taproot system.
Inflorescence	axillary, sometimes terminal spike or cyme up to 3cm long or flowers solitary; bracts ovate, often with long and spinous micro flowers.
Flower	unisexual, regular, tetramerous, usually sessile, small, axillary, greenish white.
Sepal	4 toothed calyx campanulate, 2–4mm long, with triangular lobes;
Petal	4, linear-oblong to oblong, greenish to yellowish, the upper part reflexed over the calyx
Androecium	male flowers with stamens inserted at the base of the rudimentary ovary, exerted; female flowers with staminodes
Gynoecium	superior ovary, up to 4.5mm long with a broad sessile stigma
Seed	Disc shaped, Black
Fruit	Globose, berry, greenish-white. Edible

Table 2. Fluorescent analysis of *Azima tetracantha*

Powder + Reagent	**Visible (400- 800 nm)**	**U.V short (254 nm)**	**U.V long (366 nm)**
Powder as such	Green	Black	Green
Powder + 1 N NaOH in H_2O	Black	Black	Dark green
Powder + 1N HCL	Black	Black	Dark green
Powder + 50% HNO_3	Dark black	Black	Green
Powder + Iodine solution	Dark black	Black	Green
Powder + Acetic acid	Yellow	Black	Green
Powder + 5% $FeCl_3$	Brown	Black	Bright green
Powder + Con.H_2So_4	Brown	Black	Bright green
Powder +Con. HCl	Brown	Black	Bright green
Powder + 95% Alcohol	Yellow	Black	Green

Table 3. Physiochemical characters of *Azima tetracantha*

Parameters	Values
Foreign matter	2.4
Loss on drying	9%
Swelling index	3.79%
Foaming index	>100 Units
Ph	7.25

Table 4. Phytochemicals tested in *Azima tetracantha*

Sl.No	Phytochemicals	Present/Absent
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Alkaloids	+
5	Terpenoids	+
6	Phlobatannins	+
7	Glycosides	-
8	Simple phenolics	+
9	Coumarins	-
10	Quinones	-
11	Acids	+
12	Flavanols	+
13	Lignin	+
14	Steroids	+
15	Gums and mucilage	-

Table 5. Quantitative estimation of *Azima tetracantha*

Phytochemicals	Amount(mg/g)
Alkaloids	0.40
Steroids	10.4
Phenols	85

Table 6. ABTS antioxidant assay

SL. No	Concentration of extract	Trolox (percentage of inhibition)	Plant extract (percentage of inhibition)
1	20µg/ml	41%	37%
2	40µg/ml	62%	59%
3	60µg/ml	88%	74%
4	80µg/ml	98%	96%

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