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Research Article

PHARMACOGNOSTIC EVALUATION OF AERIAL PARTS OF *ALYSICARPUS MONILIFER L.(DC.)*

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ABSTRACT

Alysicarpus monilifer (Papilionaceae) is traditionally utilized in various ailments like inflammation, pain, skin diseases, jaundice and fever. The present study was aimed at pharmacognostic and preliminary phytochemical evaluations of aerial parts of *Alysicarpus monilifer* to develop its monograph. Pharmacognostical investigations were carried out in terms of macroscopical and microscopical characters, physio-chemical constants, extractive values in different solvents, fluorescence analysis of dry powder- its reaction after treatment with chemical reagents under visible light, and UV light at 254 nm and 366 nm. The dried root powder was subjected to successive Soxhlet extraction was carried out using petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. These solvent extracts were subjected to preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, steroids, glycosides, alkaloids, tannins and phenolic compounds. The phytochemical evaluation revealed the presence of carbohydrates, phytosterols, triterpenoidal saponins, fixed oils, phenolics and tannins.

Keywords: *Alysicarpus monilifer*, Aerial parts, Pharmacognosy, Preliminary phytochemistry

INTRODUCTION

Natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates and its importance in modern medicine has been discussed in different reviews and reports [1].

Alysicarpus monilifer L.(DC.) (Papilionaceae), commonly known as *Samervo* (Gujarati) or *Juhi ghas* (Hindi), is a turf forming legume and native to Africa and Asia. In India it is distributed throughout the plains- Madras, Jammu, Bombay, Punjab, Gujarat- except Kutch and Bulsar, Madhya Pradesh and Uttar Pradesh. It is a prostate, procumbent or decumbent perennial herb; stem of which is around 12- 60cm long, woody at the base. It is a branched; branches are terete clothed with covering trichomes. The herb is up to 50cm in length and hairy when young [2,3]. *Alysicarpus monilifer* has been used in indigenous system of medicine as anti-inflammatory and in stomach-ache [4], an antidote to snake bite [5,6]. It is also used in skin diseases and as a diuretic [7,8]. The leaves are used in fever [9] and jaundice [10].

Herbs show a number of problems when quality aspect is considered because of the nature of the different secondary metabolites present therein, variation in the chemical profile of herbs due to intrinsic and extrinsic factors like growth, harvesting, geographical source, storage, drying conditions etc [11]. To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential, the first step towards it is authentification followed by creating numerical values of standards for comparison [12]. Pharmacognostical parameters like macroscopy, microscopy, quantitative leaf microscopy, fluorescence analysis, physicochemical and phytochemical studies are few of the basic parameters for standardization of herbals. Hence, in present work, establishment of the pharmacognostic and phytochemical profile of the aerial parts of *Alysicarpus monilifer* is carried out, which will assist in standardization, can guarantee quality, purity and also be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Collection of plant material

Fresh and fully grown plants of *Alysicarpus monilifer* were collected from New Vallabh Vidyanagar (beyond G.I.D.C. phase IV) in the month of September and identified by Dr. Bhanu H. Kakrani, Lecturer, Dept. of Botany, Tolani College of Arts & Science, Adipur (Kutch) and its voucher specimen deposited (PHK/Am-1/1/ARGH-11) with Department of Pharmacognosy, A. R. College of pharmacy & G.H. Patel Institute of Pharmacy, Vallabh Vidyanagar.

Pharmacognostic Evaluation

Macroscopy: The macroscopic characters such as color, odour, taste, nature, texture were studied for morphological investigation. The shape, apex, base, margin, taste and odour were determined in case of leaves [13].

Microscopy [14]

Preparation of transverse section of leaf and stem

The T.S. of leaf and stem were cleared of coloring matter by heating with chloral hydrate. After clearing, the set of slides were mounted in glycerin. Another set of sections was stained with phloroglucinol and concentrated hydrochloric acid (1:1) mixture for differentiating lignified tissues. A third set of sections was treated with dilute iodine solution. All the sections were then observed under 10X and 45X.

Surface preparation of the leaf

Small portion of leaf (2 mm square) was placed in chloral hydrate solution in test tube; epidermis was exposed by scrapping of the tissues with sharp edge of razor on the glass slide. Water was added slowly and continuously scrapping was done till transparent. The portion was mounted in a mixture of equal portion of glycerin.

Powder preparation of leaves and stem

The aerial parts of *Alysicarpus monilifer* were dried under shade. The plant parts were powdered by grinding and passed through the sieve number 60. Finally, from this coarse powder, microscopical examination was done. Slides were prepared in same manner as mentioned in above method.

Quantitative microscopy [15,16]

Quantitative leaf microscopy to determine palisade ratio, stomatal number, stomatal index, palisade ratio, vein islet number and vein termination number were carried out on epidermal strips. Other quantitative microscopical parameters are determination of fibre size in powder of aerial parts.

Fluorescence analysis [17]

Powdered leaf material was subjected to analysis under ultra violet light after treatment with various chemicals and organic reagents.

Proximate analysis [15,16,18]

The ash values, extractive values, moisture content, crude fibre content, swelling index, elemental analysis, foaming index were

performed according to the official methods prescribed in Indian Pharmacopoeia and the WHO guidelines on quality control methods for medicinal plant material

Phytochemical Analysis

The dried powdered plant material was successively extracted with the solvents of increasing polarity in a Soxhlet apparatus utilizing petroleum ether (60 - 80), Toluene, acetone, chloroform, methanol and water. The liquid extracts obtained with different solvents were collected and the consistency, color, appearance of the dried extracts and their percentage yield were noted. The extracts obtained from powder by successive solvent extraction were subjected to qualitative examination for the phytoconstituents like alkaloids, glycosides, carbohydrates, phytosterols, fixed oils, saponins, phenolic compounds, tannins and flavonoids, proteins and amino acids by the reported methods [14,19,20].

RESULTS AND DISCUSSION

Macroscopy

Herb (Fig. 1)

- Prostate
- Procumbent or decumbent
- Stem: 12- 60cm long, woody at the base.
- Branched, branches terete clothed with spreading trichomes, upto 50cm in length, hairy when young.

Fig. 1: *Alysicarpus monilifer* in natural habitat

Fig. 2: *Alysicarpus monilifer* leaf morphology

Leaves (Fig. 2)

- Unifoliate
- Type: simple
- Shape: Elliptical- oblong or ovate.
- Size: 0.4 to 2.5cm in length, 0.2-1.2cm broad.
- Surface: glabrous on upper surface, sparingly hairy on nerves on lower surface
- Margin: ciliate
- Apex: obtuse/ mucronate
- Base: obtuse, often cordate, symmatrical
- Petiole: 3-5mm long.
- Stipules: 3-4 mm long, scarious, striate, lanceolate, and acute.

Inflorescence (Fig. 3)

- 4 to 8 flowered
- Terminal & axillary racemes
- Color: reddish purple & pink
- Size: up to 5mm long
- Calyx lobes: triangular, upto 4 mm long, linear lobes usually shorter
- Corolla: as long as or slightly more than calyx.

Fruits/ Pods (Fig. 4)

- Up to 1.6cm in length
- Moniliform
- 3-6 jointed; joints hairy, reticulately veined.

Fig. 3: Terminal raceme of *Alysicarpus monilifer*

Fig. 4: Pods of *Alysicarpus monilifer*

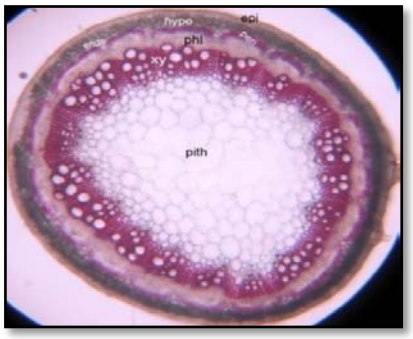
Microscopical Characteristics

Transverse section of stem

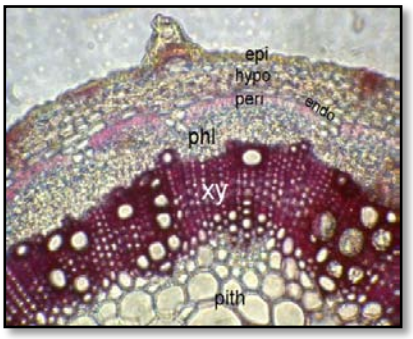
A Transverse Section of stem of *Alysicarpus monilifer* is circular in outline having numerous unicellular covering trichomes (Fig. 5c). It is dicot stem**.** 2 to 3 layered **epidermis** is present in the outermost region with well defined cuticle extending over it. **Hypodermis** is present which is made up of collenchymatous tissue forming a zone of 5 to 6 layers of tangentially elongated cells (Fig. 5a). **Endodermis** present is followed by schlerenchymatous **pericylcic fibres** which are lignified, forming 3 to 4 layered bundle cap. **Vascular bundles** are arranged in a ring forming a continuous cylinder due to activity of inter-fascicular cambium (Fig. 5b). **Phloem** lies externally, consisting of sieve tubes, companion cells, phloem parenchyma and phloem fibres. Phloem is followed by lignified elements of xylem. **Xylem** consists of xylem fibres, reticulate xylem vessels, trachieds and xylem parenchyma. Inner to xylem big parenchymatous **pith** is present. Prisms of calcium oxalate crystals and starch grains (Fig. 5d) are present in the pith region.

Transverse section of leaf

A Transverse Section of leaf shows typical dorsiventral leaf. Both sides are covered by upper and lower epidermal layers (Fig 6a). **Mesophyll** is divided into palisade and spongy parenchyma. **Palisade** constitutes about 2-3 layers below the upper epidermis, cells of which are tubular, radially arranged at right angle to the upper epidermis. It contains numerous chloroplasts. **Spongy parenchyma** is towards lower epidermis and its cells are loosely arranged. **Vascular bundle** in the midrib is larger in size than in the wings. Vascular bundle is bicollateral (Fig. 6b).A distinct parenchymatous bundle sheath surrounds all the bundles and in a bigger bundle schlerenchymatous patches occur at both the poles. **Unicellular trichomes** are present on the lower epidermis.

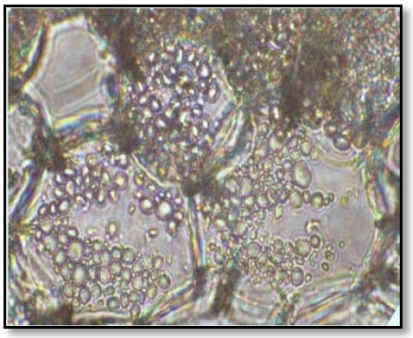


(a) Entire T. S.



(b) Detailed T.S.

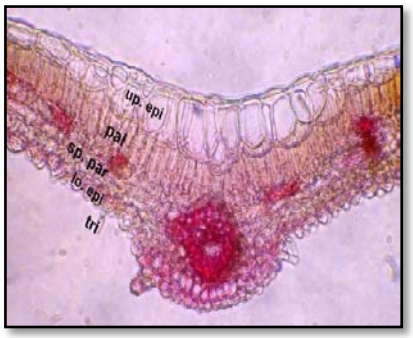
(c) Trichomes



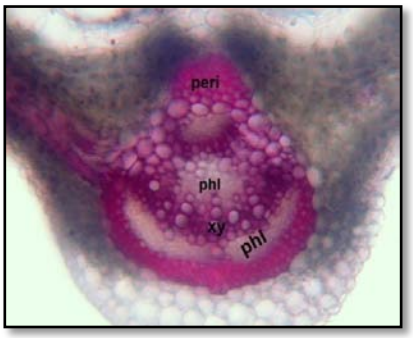
(d) Starch grains

Fig. 5: Transverse Section of Stem of *Alysicarpus monilifer*

epi- epidermis; hypo- hypodermis; endo- Endodermis; peri- pericycle; phl- ploem; Xy- xylem



(a) Entire T.S



(b) Vascular Bundle

Fig. 6: Transverse section of leaf of *Alysicarpus monilifer*

Up. epi- upper epidermis; pal- palisade cells; sp. par- spongy parenchyma; low. epi- lower Epidermis; tri- unicellular trichomes; Xy- xylem; ph- phloem; peri= pericycle

Surface preparation of leaf

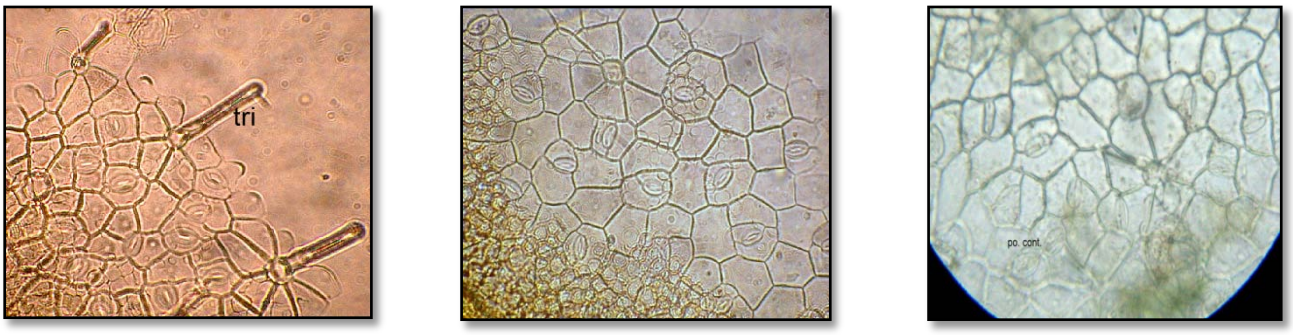
In the surface view of *Alysicarpus monilifer* leaf, the lower epidermal cells (Fig 7a) show the presence of irregular epidermal cells, anisocytic and paracytic stomata and simple, unicellular covering trichomes. In the surface view of upper epidermis (Fig. 7b), characteristics noted were irregular epidermal cells and paracytic stomata.

The surface preparation of *A. monilifer* shows "**Stomatal clustering**", an abnormal stomatal pattern formed by two or more stomata in leaf epidermis (Fig. 7c). The stomatal clustering is considered a new marker for environmental perception and adaptation in terrestrial plant. The drought and salt stresses significantly increase the stomatal density and stomatal index and similarly stomatal clustering is also raised due to the same factors. The stomatal clustering can be classified into:

- Contiguous clustering
- Non- contiguous clustering

The plant here shows 2-4 polarly contiguous stomata.

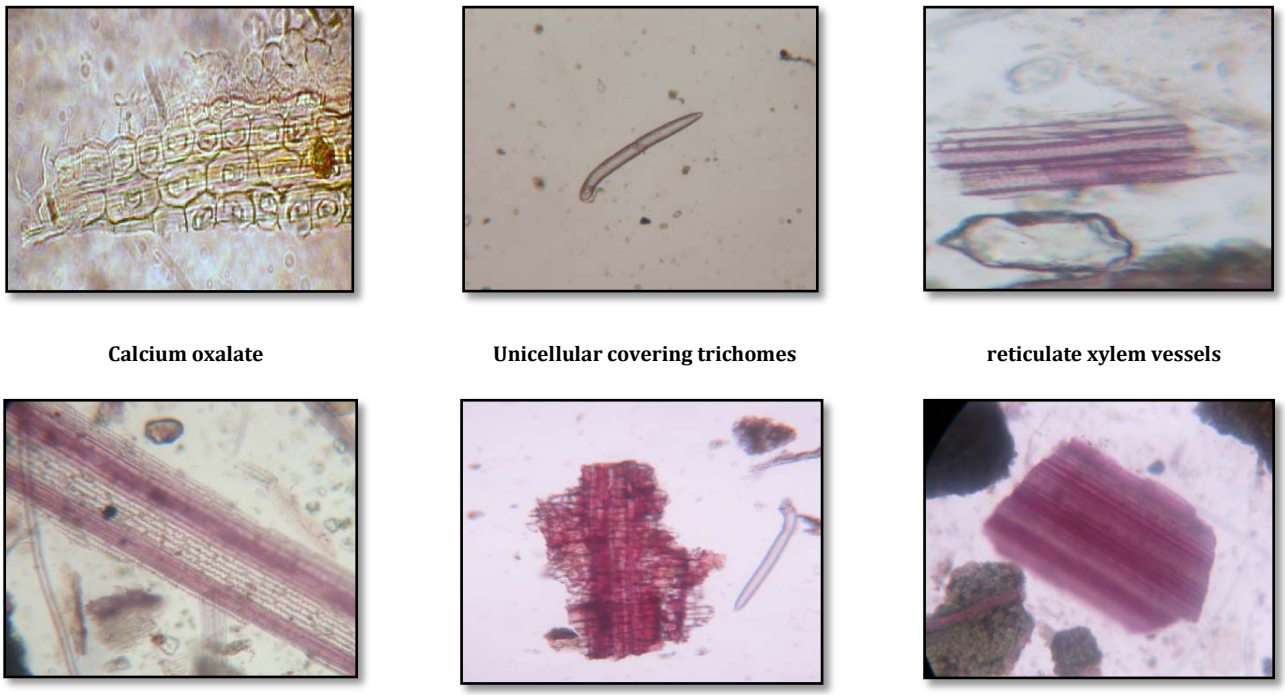
3.2.4 Powder analysis of aerial parts of the plant: The powder characters of a drug are mainly used in the identification of the drug in the powder form. The leaf powder was light green in colour with strong and characteristic taste. On microscopical examination, the powder showed calcium oxalate crystals, unicellular covering trichomes, lignified reticulate xylem vessels, lower epidermis of flowering glume, lignified xylem cells and wood parenchyma (Fig 8).



(a) Lower Epidermis **(b) Upper Epidermis** **(c) Stomatal clustering**

Fig. 7: Surface preparation of upper epidermis leaf of *Alysicarpus monilifer*

po. cont.= polarly contiguous



Calcium oxalate **Unicellular covering trichomes** **reticulate xylem vessels**

Lower epidermis of a flowering glume **Lignified xylem cells** **Wood parenchyma**

Fig. 8: Powder Study of aerial parts of *Alysicarpus monilifer*

Quantitative Microscopy

Quantitative microscopic data are found to be constant for a species. These values are especially useful for identifying the different species of genus and also helpful in the determination of the authenticity of the plant

Determination of fibre size

The width and length of fibres of *Alysicarpus monilifer* aerial parts were found to be as indicated in Table 4

Proximate Analysis

Proximate Analysis of powder of *Alysicarpus monilifer* is given in following table 3:

Total ash values of leaf indicate amounts of carbonates, phosphate, silicates and silica in leaf powder. Acid insoluble value indicated presence of silica especially sand and siliceous earth. Water soluble ash values indicate amount of sugar and salt present. Water soluble extractive values of leaf was found to be higher than alcohol soluble extractive value, however value indicated that most of the phytoconstituents present in drugs are soluble in polar solvents. Moisture content reveals the percentage of moisture present in the drug. The value of foaming index indicates the presence of saponins in the plant.

Fluorescence Analysis

The treatment of powdered drug with different chemical reagents reveals the presence of different chemical constituents present in the powdered drugs. Fluorescence analysis reveals the presence of chemical constituents with fluorescence character in UV light and color change in observed in the visible light.

Elemental Analysis

The element content of aerial parts of *Alysicarpus monilifer* was determined by Inductive coupled plasma. It showed absence of heavy metals.

Phytochemical Analysis

Preliminary profiles of Successive solvent extracts

The dried leaves are extracted with petroleum ether, toluene, chloroform, acetone, methanol and water. The consistency, color, appearance of the extracts and their percentage yield are given in table no. 6.

Preliminary phytochemical screening

All the above extracts were tested with various reagents and the results for the same are reported in table no.7. The various extracts showed the presence of phytosterols, carbohydrates, phenolic compounds, tannins, fixed oils and mucilage.

Total Phenolic Content

By extrapolating absorption value in the above calibration curve of the standard gallic acid, total phenolic content was found to be 7.016 mg Gallic acid equivalents (GAE) per 100 mg of powder extract.

Table 1: Quantitative Microscopy of Leaf of *Alysicarpus monilifer*

Sr. No.	Determination	Values per Square mm
1	Stomatal number	
	a) Upper surface	220- 250
	b) Lower surface	423- 550
2	Stomatal Index	
	a) Upper Surface	17-21.2
	b) (b) Lower Surface	13.1- 20.4
3	Vein-Islet Number	18- 26
4	Veinlet Termination Number	43.75- 62.5
5	Palisade Ratio	
	a) Upper Surface	5.5- 10
	b) (b) Lower Surface	4- 7.4

Table 2: Fibre length and width of aerial parts powder

1	Length	203.68- 360- 524 µ
2	Width	8- 16- 24 µ

Table 3: Proximate Analysis of Leaves of *Alysicarpus monilifer*

Sr. No.	Standardization Parameter	Value
1	Total Ash	13.4 % w/w
2	Acid Insoluble Ash	4.2 % w/w
3	Water Soluble Ash	2.8 % w/w
4	Alcohol Extractive Value	5.04 % w/w
5	Water Extractive Value	16.56 % w/w
6	Foaming index	100
7	Moisture Content	5.46%
8	Total solid content	94.54%
9	Swelling index	1.69

Table 5: Elemental analysis of aerial parts of *Alysicarpus monilifer*

Element	Wavelength (nm)	Instrument Detection Limit	Sample Detected
Zinc (Zn)	206.200	0.0059	15.764 mg/kg
Arsenic (As)	193.696	0.0530	Not Detected
Lead (Pb)	220.353	0.0420	Not Detected
Selenium (Se)	196.026	0.0750	Not Detected
Cadmium (Cd)	228.802	0.0027	0.4207 mg/kg

Table 4: Fluorescence analysis of leaf powder of *Alysicarpus monilifer*

Reagents	Day light	UV (254 nm)	UV (365nm)
Picric acid	Yellowish green	Green	Brown
Ammonia soln	Light green	Green	Light brown
1N NaOH (Aq.)	Yellowish green	Green	Brown
1N NaOH(Alc.)	Green	Green	Brown
1N HCl	Greenish brown	Light green	Brown
5% Iodine soln	Brownish green	Green	Brown
5 % $FeCl_3$	Brown	Green	Brown
1N H_2SO_4	Greyish green	Greenish blue	Grey
Acetic acid	Green	Green	Brown
Dil. HNO_3	Grey	Greyish green	Brown
50% HNO_3	Brown	Green	Brown
10%Potassium dichromate	Yellowish brown	Green	Brown
Methanol	Green	Green	Brownish red

Table 8: Preliminary Profile of Successive solvent extracts of Aerial parts of *Alysicarpus monilifer*

Sr. No.	Solvent	Color & Consistency (After Drying)	Average Value of Extractive (% w/w)
1	Petroleum Ether (60º - 80ºC)	Dark green & Sticky	2.565 %
2	Toluene	Greenish black & Sticky	2.17 %
3	Chloroform	Dark Green & Nonsticky	2.91 %
4	Acetone	Greenish black & Nonsticky	1.69 %
5	Methanol	Brown & Sticky	8.96 %
6	Water	Dark brown & Non-sticky	12.91 %

Table 9: Phytochemical Screening

Sr. No.	Constituents	Pet. Ether	Toluene	Chloroform	Acetone	Methanol	Water
1.	Phytosterol	+	+	+	-	-	-
2.	Triterpenoids	+	+	+	-	-	-
3.	Saponins	-	-	+	-	+	+
4.	Tannins and phenolics	-	-	-	-	+	+
5.	Carbohydrates	-	-	-	-	+	+
6.	Fixed oils	+	+	-	-	-	-
7.	Mucilage	-	-	-	-	-	+
8.	Flavonoids	-	-	-	-	-	-
9.	Glycosides	-	-	-	-	-	-
10.	Alkaloids	-	-	-	-	-	-

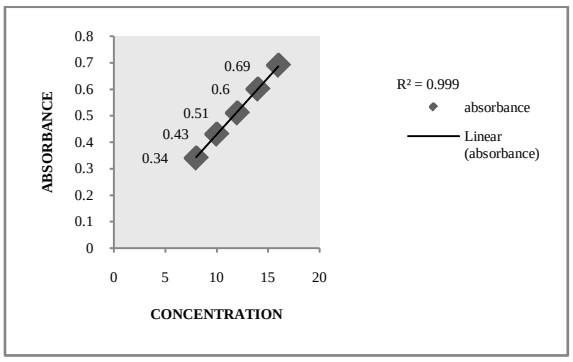


Fig. 9: Graph of absorbance versus concentration of standard gallic acid

CONCLUSION

The present study pertains to detailed pharmacognostical and phytochemical investigation of *Alysicarpus monilifer*. Macro- and microscopical characters, behavior of powdered drug on treatment with different chemical reagents, fluorescence analysis, extractive values, ash values and preliminary phytochemical tests were carried out to study the distinctive features of the drugs. Such parameters provide basis for standardization/characterization of genuine drug. Transverse section of leaf of *Alysicarpus monilifer* shows it is dorsiventral type of leaf. Leaf showed presence of 2-3 layers of palisade cells below upper epidermis, bicollateral vascular bundle and a number of unicellular covering trichomes on the lower epidermis. In surface view it shows the presence of anisocytic type stomata.

The surface preparation of *A. monilifer* shows "Stomatal clustering", an abnormal stomatal pattern formed by two or more stomata in leaf epidermis, particularly polarly contiguous stomatal clustering. Transverse section of stem of *Alysicarpus monilifer* shows it is circular in outline, possessing well defined cuticle over epidermis, followed by hypodermis, endodermis, schlerenchymatous pericyclic

fibres, phloem, xylem and a large pith with calcium oxalate crystals and starch grains in it. It also possesses numerous unicellular covering trichomes. Powder study of aerial parts of *Alysicarpus monilifer* showed presence of unicellular covering trichomes, calcium oxalate crystals, reticulate xylem vessels, upper epidermis of flowering glume, wood parenchyma and porions of lignified xylem parenchyma. Preliminary phytochemical screening of the extracts revealed that the plant contains carbohydrates, tannins, phenolic compound, steroids, saponins, mucilage and fixed oil.

These parameters, which are being reported for the first time in this way for *A. monilifer* aerial parts, could be useful as quality control parameter. Any crude drug which is claimed to be *A. monilifer* but whose characters significantly deviate from the character above would then be rejected as contaminated, adulterated or downright fake.

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