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Original Article
Standardization and Quality Control

Pharmacognostical Evaluation of an Extra-pharmacopoeial Drug of Ayurveda - *Anisochilus carnosus* (L.f.) Wall.

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ABSTRACT

Introduction: *Anisochilus carnosus* (L.f.) Wall., locally known as *Arikal tumbe* by traditional practitioners of Udupi, is an aromatic herb of Lamiaceae. Leaves and aerials parts are used in medicine by traditional practitioners in the treatment of gastric ulcers and stomach ache. ***Methods:*** Mature whole plant of *A. carnosus* (L.f.) Wall. after flowering and fruiting was collected from Udupi District and authenticated. Macro-microscopic, physicochemical standards, HPTLC and secondary metabolites screening were recorded scientifically. ***Results:*** The pharmacognostic study of leaf has shown single layer upper epidermis with cuticle cyst walls, covering of trichomes which are uniserate with blunt apex. Anatomical features of Stem of *A. carnosus* exhibited trichomes, which were attached to epidermis, beneath these 2 or 3 layers compactly arranged collenchymas was present. In transverse section root showed outer cork which was thick wall brown in colour followed by cortex 5-6 layers. Powder microscopy characteristics showed the presence of starch in parenchyma region. Physicochemical standards and HPTLC represent the standard out prints of the drug. Preliminary Phytochemical study reveals it contain it Alkaloids, Carbohydrates, Steroids, Tannins, Phenol, Carboxylic acid and Quinone. ***Conclusion:*** Pharmacognostical study carried out on *A. carnosus*, showed quality standards of the drug, with respect to its macro-microscopy, physico-chemical standards and HPTLC.

KEYWORDS

Arikal tumbe, HPTLC, Macro-microscopic, physicochemical standards.

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INTRODUCTION

India is a rich treasure trove of diverse flora along with ethno-medicinal knowledge. Ethno medicine is the study or comparison of the traditional medicine practiced by various ethnic groups.[1] Traditional knowledge encompasses wisdom, knowledge, teaching and experience of these communities and many a times it is orally transmitted from generation to generation.[2] Urbanization and changed life style resulted in slow erosion of such knowledge rays. India with huge repository of such knowledge must collect and scientifically document these information to enrich its existing Pharmacopeia.[3]

Anisochilus carnosus (L.f) Wall. is an aromatic herb, belonging to family Lamiaceae commonly known as *Arikal tumbe* by traditional practitioners of Udupi district.[4] It is an annual herb which grows at high altitudes of Western Ghats, fairly common in the crevices of exposed rocks.[5] It is 30 to 60 cm tall, rough, branched plant with leaf stalks upto 1.3 to 5 cm long. Leaves are ovate-oblong, velvety in nature having crenulate margins, inflorescence is a verticillaster. Leaves and aerials parts are used in medicine by traditional practitioners in the treatment of gastric ulcers and stomachache. The leaves are also kept between toes to cure fungal infections of foot and toes.[6] Till now the quality standards of this multiple beneficiary plant has not been documented. With this background, a detailed pharmacognostic study of different parts of the drug employing macro-microscopy, physicochemical standardisation, secondary metabolite screening and HPTLC fingerprinting has been planned in this communication.

MATERIALS AND METHODS

Collection and authentication

Mature whole plant of *A. carnosus* (L.f.) Wall. flowering and fruiting was collected from Udupi District. Morphology features were compared with Flora of Udupi and a reference sample is deposited at Pharmacognosy Unit of SDM Centre for research in Ayurveda and Allied Sciences Udupi (Vouncher No: 16081201). Plant parts were cleaned from extraneous matter, washed properly in slow tap water before shade drying of root, stem and leaves. After complete air drying the plant material was powdered and preserved for further study. Some fresh samples were preserved in FAA solution for microscopic study.[7]

Macroscopic evaluation

Morphological characters of root, stem and leaves were studied by visual observation following the Standard protocols. These samples were keenly observed under naked eye to record the specific botanical characters and it was also recorded using Canon digital camera with size indicating rulers.[8]

Microscopic evaluation

The histology of root, stem and leaves was recorded following standard procedures. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnification of the figures was indicated by the scale-bars.[9]

Powder microscopy

A pinch of powder was warmed with drops of chloral hyadrate on a microscopic slide and mounted in glycerin. Slides were observed under the microscope, diagnostic characters marked and photographed with Zeiss Axio Cam camera under bright field light.[10]

Physico-chemical standards

A.carnosus whole plant powder was tested for pharmacopoeial constants like loss on drying at 105°C, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive as per standard protocol.[11]

Preliminary phytochemical analysis

Preliminary phytochemical investigation was done to detect the presence of alkaloids, steroids, carbohydrates, tannin, flavinoids, saponins, triterpenoids, coumarins and phenols in ethanololic extracts of *A. carnosus*.[12]

HPTLC finger printing

One gram of *A. carnosus* Whole plant powder was extracted with 10 ml of ethanol. Four, 8 and 12 µl of the above extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in toluene: ethyl acetate (7.0: 1.0) mobile phasse. The developed plates were visualized under short and long UV and scanned under UV 254nm, 366nm and 620nm before derivatisation with vanillin sulphuric acid. R_f, colour of the spots and densitometric scan were recorded.[13,14]

RESULTS AND DISCUSSION

A. carnosus is an aromatic semisucculent annual herb. Thick tap root, with brownisy white cork layer and aromatic odour. Stems are robust and branched. Leaves 2 to 5 x 2 to 3.5 cm broadly ovate-oblong to circular, base cordate, tip blunt to rounded, petiolate, with crenulate border and a velvety appearance (Fig. 1).

Figure 1. Macroscopy of *Anisochilus carnosus* (L.f) Wall.

1.1 Habit

1.2 Leaf

TS of root

In transverse section root showed outer cork which has thick wall and brown in colour followed by 5 to 6 layerd cortex. Rosette crystals are seen in the cortical region. Vascular bundle is collaterally closed. Large xylem vessels, surrounded by xylem rays are seen. Compared to cortical region, pith region showed big parenchyma cells (Fig. 2).

TS of stem

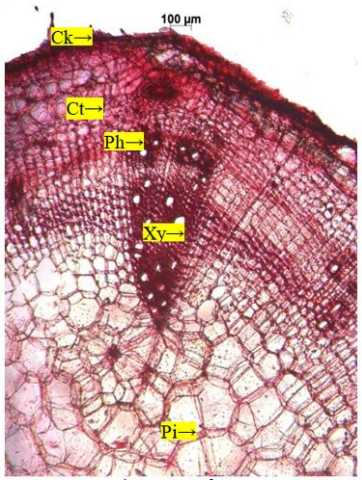
TS of stem showed trichomes attached to epidermis, beneath which 2 to 3 layers of compactly arranged collenchyma cells are seen. There is a layer of pericycle below the cortex which is mainly composed of parenchyma cells containing starch grain. Endodermis single layered below which there was a conjoint collaterall closed vascular bundle with xylem on the inner side and phloem on the outer side. Pith consisted of bigger parenchyma cells with thick walls (Fig. 3).

TS of leaf

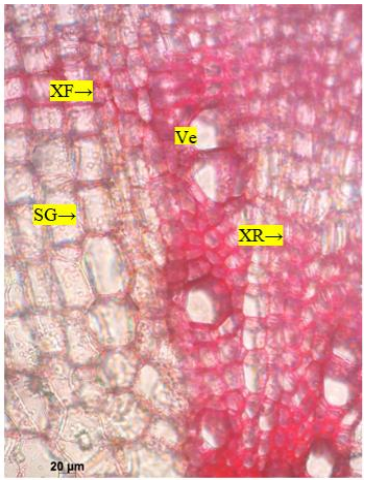
Transverse section of the leaf showed upper epidermis of single layer with cuticle walls and covering type of uniseriate trichomes with blunt apex. Mesophyll was differentiated into palisade and spongy parenchyma. Single layered palisade parenchyma made up of radially elongated cells present. Spongy parenchyma had many layered loosely arranged cells.

Lower epidermis shows numerous trichomes which are unicellular, multicelluar and glandular. In midrib, there was a strip of collenchyma, present below the upper and lower epidermii. This is followed by cortical parenchyma containing crystals of calcium oxalate embedded in the form of rosette crystals. Bicollateral vascular bundle with xylem in the centre and phloem on both the sides are present (Fig. 4).

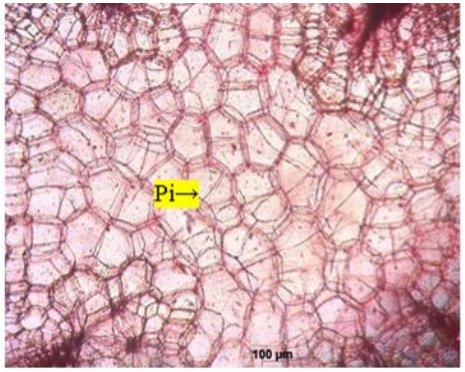
Figure 2. Microscopy of Root of *Anisochilus carnosus* (L.f.) Wall.



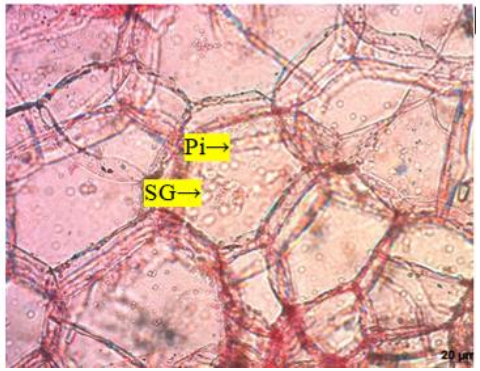
2.1 TS of root



2.2 Xylem region enlarged



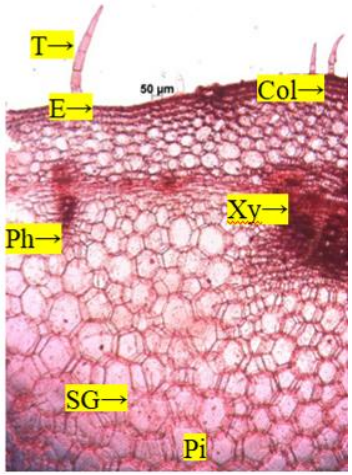
2.3 Pith region



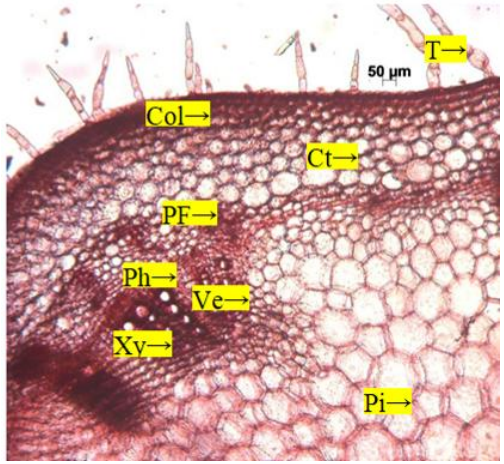
2.4 Pith region enlarged

Ck – cork; Ct – cortex; Pa – parenchyma; Ph – phloem; Pi – pith; RC – rossette crystals; ; SG – starch grains; Ve – vessel; Xy – xylem; XF – xylem fibres; XR – xylem ray.

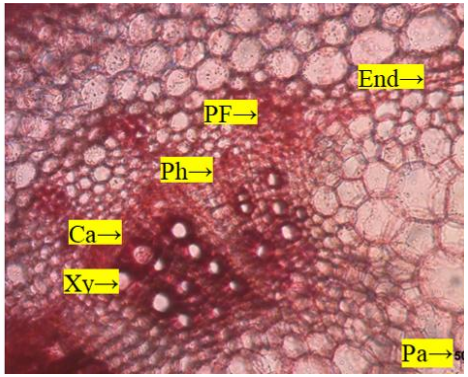
Figure 3. Microscopy of Stem of *Anisochilus carnosus* (L.f.) Wall.



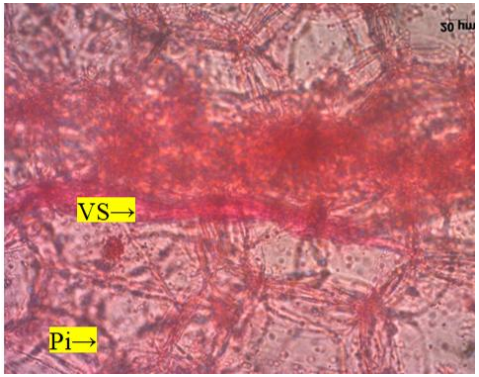
3.1 TS of stem



3.2 Outer region enlarged



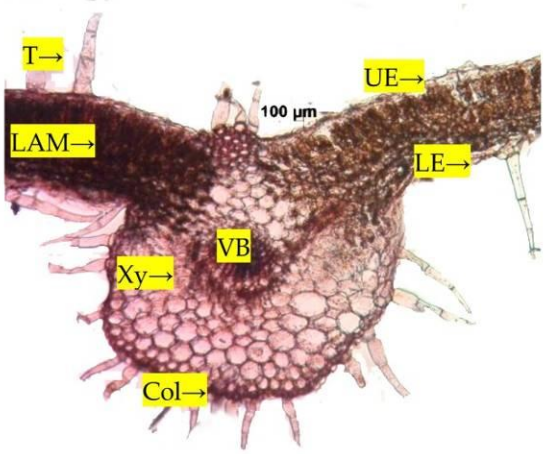
3.3 Xylem and pith



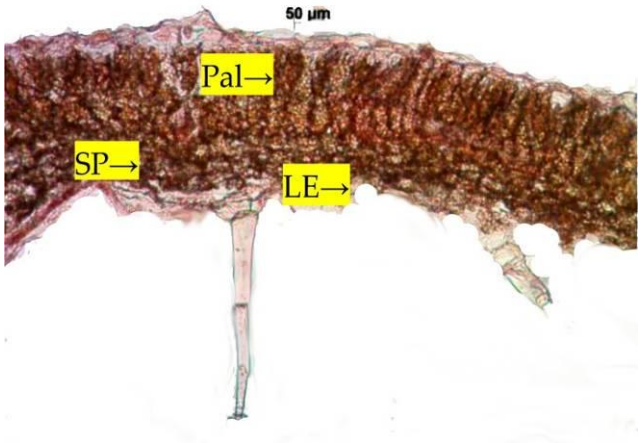
3.4 Pith

Ca – cambium; Col – collenchma; Ct – cortex; End – endodermis E – epidermis; PF – pericyclic fibres; Ph – phloem; Pi – pith; SG – starch grains; T–trichomes; Ve – vessel; VS – vascular strands; Xy – xylem.

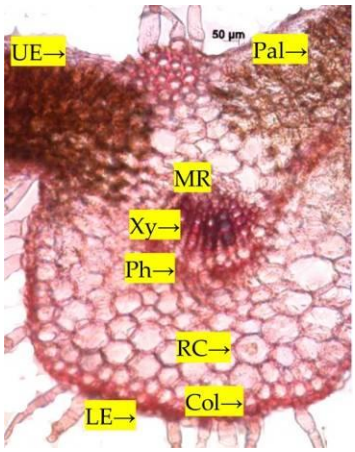
Figure 4. Microscopy of Leaf of *Anisochilus carnosus* (L.f.) Wall.



4.1. T.S of leaf



4.2. Lamina enlarged



4.3. Midrib enlarged

UE – uppr epidermis; LAM – lamina; LE – lower epidermis; MR – midrib; Pal – palisade; PF – pericyclic fibres; Ph – phloem; RC – rosette crystal; SP – spongy parenchyma; T–trichomes; VB–vascular bundle; Xy – xylem

Powder

Powder showed the epidermal cells with stomata, covering type of both uniseriate and multiseriate trichomes with blunt apex, covering type of uniseriate elongated palisade cells with chlorophyll, bundle of phloem fibres and groups of starch grains (Fig. 5).

Physico-chemical analysis

A. carnosus whole plant powder was tested for loss on drying at 105^{0}C, total ash, acid insoluble ash, ethanol and water soluble extractive as per standard protocol. Loss on drying was 15.43%w/w, total ash was 9.70%w/w, acid insoluble ash 0.30%w/w, water soluble ash 3.96%w/w, alcohol soluble extractive value 5.24%w/w and water soluble extractive value 22.18%w/w. Physicochemical standards were a representative of its purity, physical nature and chemical trait (Table 1).

Phytochemical study

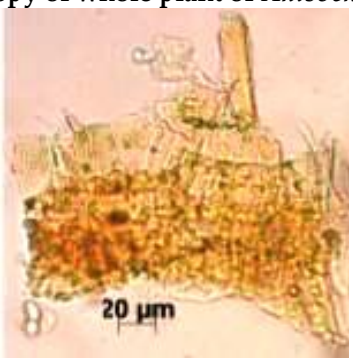
The preliminary photochemical studies are essential to know the basic constituents present in the drug. Action of any drug depends upon these basic components. Preliminary photochemical test were conducted for *A. carnosus*. Test for alkaloids (Dragendrof's test, Wagner's test, Mayer's test and Hager's test), carbohydrates (Molisch's test, Fehling's test and Benedict's), steroids (Libermann-Burchard and Salkowski), saponins, phenols, coumarin, triterpenoids, quinine, resin and tannins were conducted and result displayed(Table 2).

HPTLC

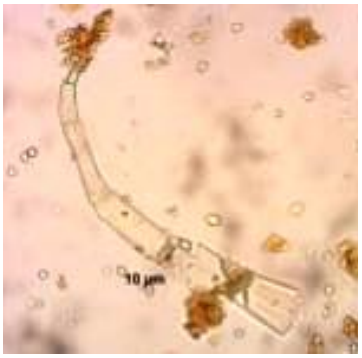
HPTLC finger print profile of ethanolic extract of *A.carnosus* has been obtained with suitable solvent system. The developed plates were visualized under UV light and white and then under light after derivation with vanillin sulphuric acid reagent. R_f, colour of the spots and densitometric scan at 254 and 366 nm were recorded. On photo documentation there were 6 spot under short UV, 13 spots under long UV and 9 spots after derivatisation with vanillin sulphuric acid reagent (Table 3 and Fig. 6). Densitometric scan at 254 nm showed 7 peaks such as 0.01 R_f,(40.78%) 0.13 R_f, (14.97%), 0.27 R_f, (4.89%), 0.40 R_f, (27.25%), 0.58 R_f, (6.70%), 0.64 R_f, (3.76%), 0.76 R_f, (1.64%) (Fig. 6). Densitometric scan at 366 nm showed 11 peak (Fig. 6) whereas at 620 nm there were 8 peaks (Fig. 6).

Figure 5. Powder microscopy of whole plant of *Anisochilus carnosus* (L.f.) Wall.

5.1 Parenchyma with starch grains

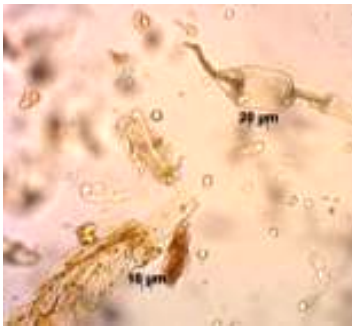


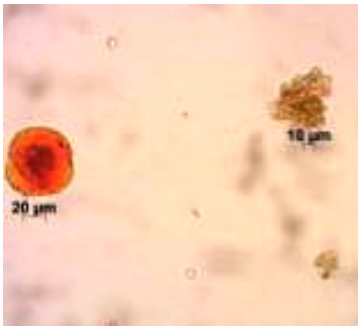
5.2 Epidermis in surface view



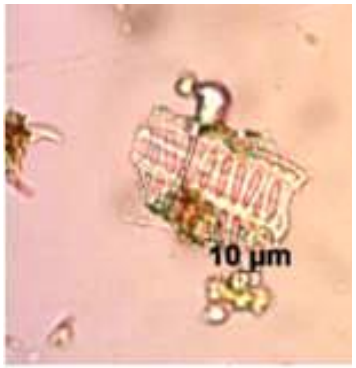
5.3 Trichome

5.4 Collapsed trichomes

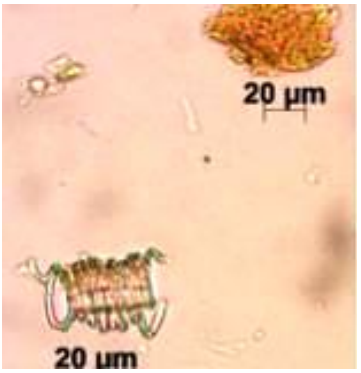




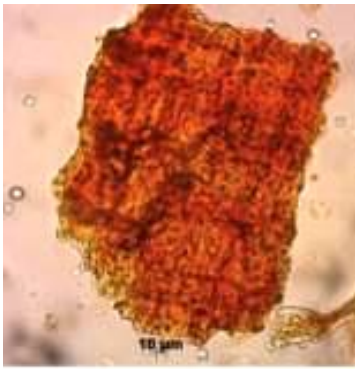
5.5 Glandular trichome



5.6 Pitted and annular vessel fragments



5.7 Spiral vessel



5.8 Mesophyll

5.9 Starch grains

5.10 Bundle of fibres

Table 1. Physicochemical standard of whole plant *Anisochilus carnosus* (L.f). Wall.

Parameter	Results n = 3 %w/w
Loss on drying	15.43
Total Ash	9.70
Acid Insoluble Ash	0.30
Water soluble Ash	3.96
Alcohol soluble extractive value	5.24
Water soluble extractive value	22.18

Table 2. Preliminary phytochemical tests of aqueous extract of whole plant *Anisochilus carnosus* (L.f). Wall.

Tests	Colour if positive	Water extract
Alkaloids		
Dragendroff's test	Orange red precipitate	Orange red precipitate
Wagners test	Reddish brown precipitate	Reddish brown precipitate
Mayers test	Dull white precipitate	Dull white precipitate
Hagers test	Yellow precipitate	Yellow precipitate
Steroids		
Liebermann- buchard test	Bluish green colour	Bluish green colour
Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer
Carbohydrate		
Molish test	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate
Tannin		
With $FeCl_3$	Dark blue or green or brown	Brown color
Flavanoids		
Shinoda's test	Red or pink	Green color
Saponins		
With $NaHCO_3$	Stable froth	No froth
Triterpenoids		
Tin and thionyl chloride test	Pink/Red color	Green color
Coumarins		
With 2 N NaOH	Yellow	Brown color
Phenols		
With alcoholic ferric chloride	Blue to blue black or brown color	Brown color
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	Brisk effervescence
Amino acid		
With ninhydrine reagent	Purple colour	Green color
Resin		
With aqueous acetone	Turbidity	No turbidity
Quinone		
Conc. sulphuric acid	Pink/purple/red	Red color

Table 3. R_f values of whole plant *Anisochilus carnosus* (L.f). Wall.

Under short UV	Under long UV	Under white light after derivatisation
-	0.08 (F. red)	0.08 (L. purple)
0.11 (L. green)	-	-
-	0.13 (F. red)	-
-	-	0.16 (L. purple)
-	0.19 (F. red)	-
-	-	0.21 (D. purple)
-	0.29 (F. red)	-
-	-	0.31 (L. purple)
-	0.33 (F. red)	-
0.39 (L. green)	0.39 (F. red)	0.39 (D. purple)
0.43 (D. green)	-	-
-	0.45 (F. red)	-
-	-	0.49 (L. purple)
0.52 (L. green)	0.52 (F. red)	-
-	0.54 (F. red)	-
0.57 (L. green)	-	0.57 (D. purple)
-	0.63 (F. red)	-
0.70 (L. green)	0.70 (F. red)	-
-	-	0.73 (L. purple)

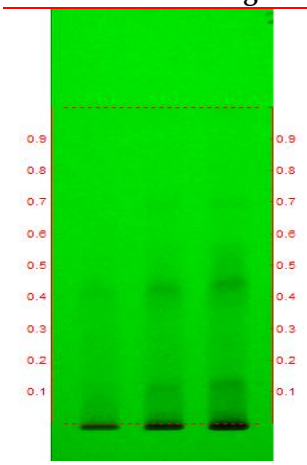
-	-	0.79 (L. purple)
-	0.86 (F. blue)	-
-	0.92 (F. red)	-

*F – fluorescent; L –light; D – dark
Solvent system – Toluene: Ethyl Acetate (7.0: 1.0)

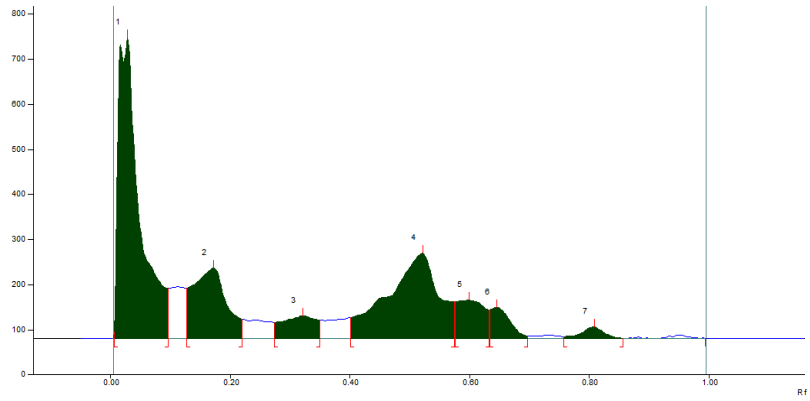
The macroscopic features recorded can be used for preliminary identification of the particular plant. In many of the earlier studies, the microscopic observation of cellular structures was found to be essential. Micorscopic recordings proved to be effective in establishing the authenticity and detection of adulteration/ substitutes for herbal raw drugs. *A. carnosus* is annual, erect herb. Microscopic features revealed the prescence of rosette crystals in the cortex region of root. Epidermal trichomes and conjoint vascular bundles are anatomical findings of stem. Bicollateral vascular bundles, strips of collenchymatous cells at midrib and epidermal unicellular, multicellular, gladlular trichomes are main features of leaf anatomy. Uniseriate and multiseriate trichomes along with groups of starch grains are marked features of powder microscopy.

Any matter other than the described parts of the drug is to be considered as foreign matter, any raw drug must be made free from foreign matter before any physico-chemical analysis is done.Total ash indicative of the total inorganic composition of the drug was found to be 9.70% w/w, acid insoluble ash indicating the silicacious matters was found to be 0.30% w/w. Water soluble ash which indicates the amount of ash which is readily soluble in water was found to be 3.96% w/w. Loss on drying indicates the moisture and volatile matter content in sample and was found to be 15.43% w/w. The solvent used for the extraction is in a position to dissolve appreciable quantities of substances likewise various solvents were used to extract these chemical constituents. The extract obtained by percolating coarse powder is indicative of approximate quantity of their chemical constituents. Alcohol soluble extractive value of the test sample was found to be 5.24% w/w and water soluble was 22.18% w/w. All these pharmacopoeial parameter helps to determine the quality and purity of herbal drugs. Preliminary phytochemical tests were conducted using the water extracts alkaloids, Steroids, Carbohydrate, Tannin, Phenols, Carboxylic acid, and Quinone. These preliminary analyses of chemical composition are one of the primary methods to study the chemistry of herbs. HPTLC photo documentation revealed presence of phyto-constituents with different R_f values. Densitometric scan of the plates showed diagnostic bands under 254 nm, 366 nm and post derivatisation. HPTLC finger printing is an effective technique of screening herbal raw drugs for authenticity and quality.

Figure 6. HPTLC of ethanolic extract of whole plant of *Anisochilus carnosus* (L.f.) Wall.

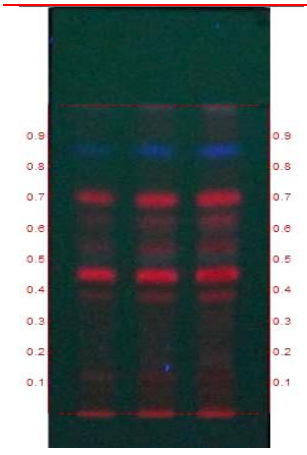


6.1 Under short UV

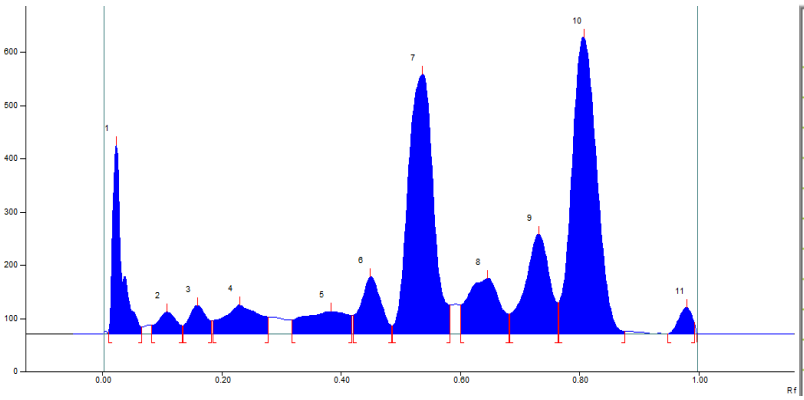


Peak	Max Position	Area %
1	0.03 Rf	40.78 %
2	0.17 Rf	14.97 %
3	0.32 Rf	4.89 %
4	0.52 Rf	27.25 %
5	0.60 Rf	6.70 %
6	0.65 Rf	3.76 %
7	0.81 Rf	1.64 %

6.2 Densitometric scan at 254 nm

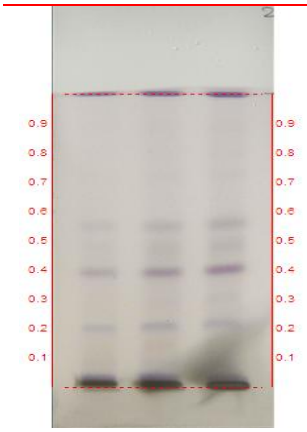


6.3 Under long UV

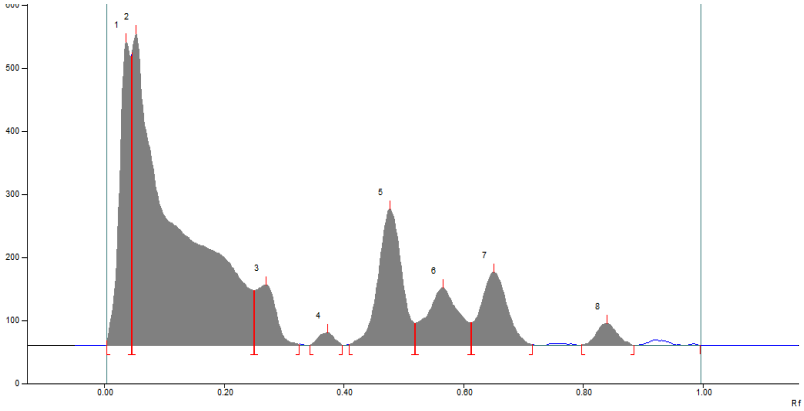


Peak	Max Position	Area %
1	0.02 Rf	7.35 %
2	0.11 Rf	1.69 %
3	0.16 Rf	2.06 %
4	0.23 Rf	4.21 %
5	0.38 Rf	4.24 %
6	0.45 Rf	4.70 %
7	0.54 Rf	26.72 %
8	0.65 Rf	7.21 %
9	0.73 Rf	10.02 %
10	0.81 Rf	30.37 %
11	0.98 Rf	1.44 %

6.4 Densitometric scan at 366 nm



6.5 Under white light (after derivatisation)



Peak	Max Position	Area %
1	0.04 Rf	13.40 %
2	0.05 Rf	52.27 %
3	0.27 Rf	4.78 %
4	0.37 Rf	0.80 %
5	0.48 Rf	12.48 %
6	0.57 Rf	7.07 %
7	0.65 Rf	7.42 %
8	0.84 Rf	1.79 %

6.6 Densitometric scan after derivation at 620nm

CONCLUSION

In Ayurvedic classics, it is advised to learn about the plants from natives and tribes, from shepherds, hermits and other experts who have well versed knowledge about these plants. Traditional knowledge is the hidden source of knowledge. *A. carnosus* (L.f) Wall. a member of *Lamiaceae* family is a less known drug, but used by the traditional Vaidyas for Finger-gap infections and other skin diseases. *A. carnosus* an annual aromatic herb with quadrangular stem commonly called as Arikal tumbe, the aerial parts of which are used in many therapeutic conditions. In transverse section root showed outer cork which was thick wall brown in colour followed by cortex 5-6 layers. Anatomical features of Stem of *A. carnosus* exhibited trichomes, which were attached to epidermis; beneath this 2 or 3 layers compactly arranged collenchymas was present. Microscopic characters of leaf shows upper epidermis single layer with cuticle cyst walls, covering of trichomes which were uniserate with blunt apex. Powder microscopy characteristics showed the presence of starch in parenchyma region. Physicochemical standards and HPTLC represent the standard outprints of the drug whereas preliminary phytochemical study of alcoholic extract showed it to possess alkaloids, carbohydrates, steroids, tannins, phenol, carboxylic acid and quinone.

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CONFLICT OF INTEREST

Nil

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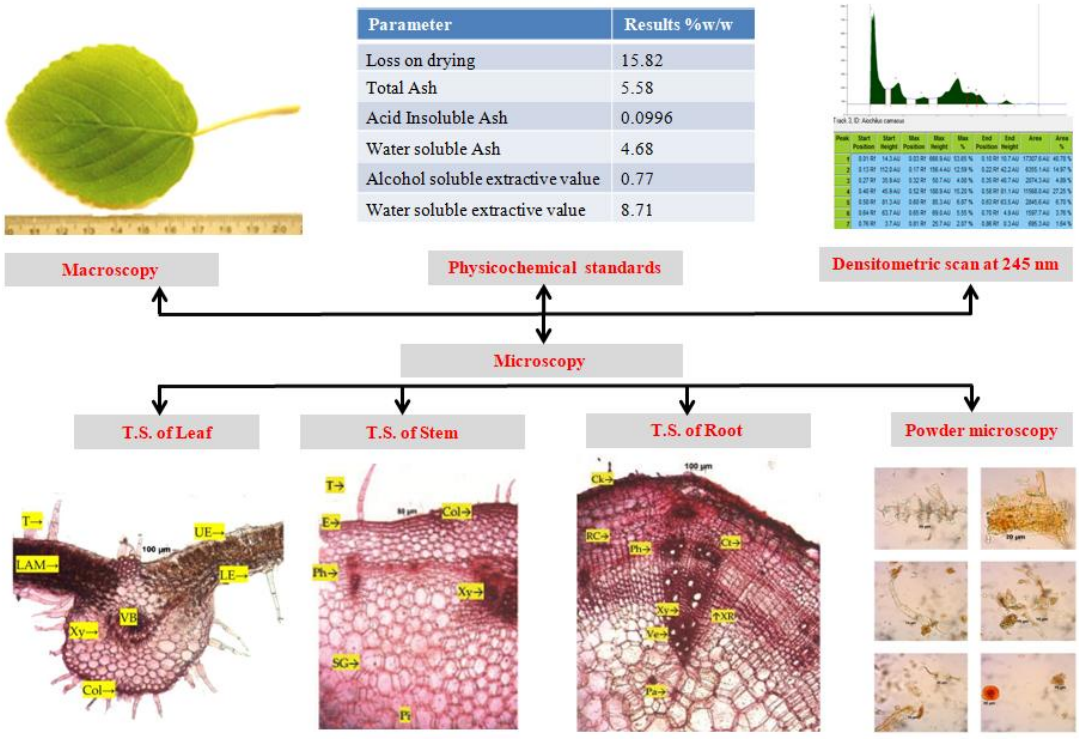
GRAPHICAL ABSTRACT



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