Journal of Traditional and Folk Practices
Volume 04(2), December 2016, 77 - 93.
ISSN 2278 - 5906

Pharmacognostic standardisation and phytochemical analysis of *Tetracera akara* (Burm. f.) Merr.

Ragesh R Nair, S R Suja*, V Vilash, A L Aneeshkumar and S Rajasekharan
Division of Ethnomedicine and Ethnopharmacology, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram 695 562, Kerala, India.
*sujasathyu@gmail.com

Received : 29 Oct 2016 Accepted: 22 Dec 2016

Abstract

Tetracera akara (Burm. f.) Merr. (Dellineaceae) is used by the Kani tribe of Kerala, India for treating various liver ailments. The plant has pharmacologically active compounds namely Betulin, Betulinic acid, Lupeol and β-Sitosterol possess anti-HIV, anti-diabetic and anti-inflammatory potential. However, there is no data available regarding the pharmacognostic standardization of this plant. Therefore, the aim of the present study was to standardize the pharmacognostic features of *T. akara* root by physicochemical characterisation, morpho-anatomical studies of various plant parts and detailed phytochemical studies by HPTLC profiling. The pharmacognostic evaluation of *T. akara* revealed the presence of characteristic morphological, organoleptic and physicochemical features of the plant. The anatomical studies helped to distinguish root and stem material even in crushed or powdered form. Detailed leaf anatomy showed the presence of uniseriate trichomes with tapered end and paracytic stomata on the lower leaf surface. Powder analysis of root showed the presence of calcium oxalate and raphide crystals, xylem fibre, vessel and tracheids. Preliminary phytochemical analysis of crude extract of *T. akara* revealed the presence of phytoconstituents like flavonoids, phenols, tannins, saponins and terpenoids. HPTLC studies of crude extract and ethanolic fraction revealed the comparative HPTLC fingerprinting profile of *T. akara* at 254 and 366nm for the first time. The present investigation thus sets a benchmark in authenticating the genuine plant material by setting diagnostic indices for the identification and preparation of monograph of *T. akara*, a new entry in the field of therapeutics.

Keywords: *Tetracera akara,* Kani tribe, HPTLC profiling.

1. Introduction

Medicinal plants have a long-standing history in the practices of traditional medicine, which is based on hundreds of years of belief and observations (Jeyaprakash *et al*., 2011). Traditionally used medicinal plants are now moving from fringe to mainstream as people are becoming more aware of therapeutic interventions of these medicinal plant resources and their products in maintaining health and preventing diseases with an eco-friendly touch. One-fourth population of the world, mainly from the developing countries depends on traditional medicines for the treatment of various ailments (Jena *et al*., 2011). A key obstacle, which has hindered the acceptance of the alternative medicines including tribal medicines in the developed countries, is the lack of documentation and stringent quality control which ensures it's safety and efficacy. In this scenario, it becomes the need of the hour to make an effort towards standardization of traditionally used medicinal plants and it can be achieved by authentication

of the correct plant source by pharmacognostic and phytochemical evaluation (Padashetty *et al*., 2008)

Tetracera akara (Burm. f.) Merr. belongs to the family Dellineaceae, is a woody climber distributed in India, Sri Lanka, China, Laos, Cambodia, Thailand, Myanmar and Indo-Malayan archipelago. In India, it is found in the Western Ghats region of Kerala and Tamil Nadu. The medicinal use of *Tetracera akara* was first reported by S. Rajasekharan and his team in 1987 during Ethnobiological studies carried out with the help of Kani tribe residing in the Pottamavu tribal settlement of Thiruvananthapuram district. The paste of the fresh root along with coconut kernal is administered orally in empty stomach, before sunrise for 3 days to cure jaundice and liver related disorders by the Kani tribe of Kerala, India (Saradamma *et al*., 1987). Leaf decoction of *T. akara* is used to treat pulmonary haemorrhages and gargles for aphthae (Udayan *et al*., 2009). It is reported that *T. akara* contains terpenoids like betulin, betulinic acid, lupeol and β-Sitosterol which have a wide range of bioactivities like anti-HIV, anti-diabetic and anti-inflammatory (Lima *et al*., 2014). However, no pharmacognostic study has been carried out on this plant and hence the objective of the present study is to evaluate various pharmacognostic properties including morpho-anatomical, microscopic, phytochemical and physicochemical characterization of *T. akara*, which will provide some useful markers for identification of crude drug.

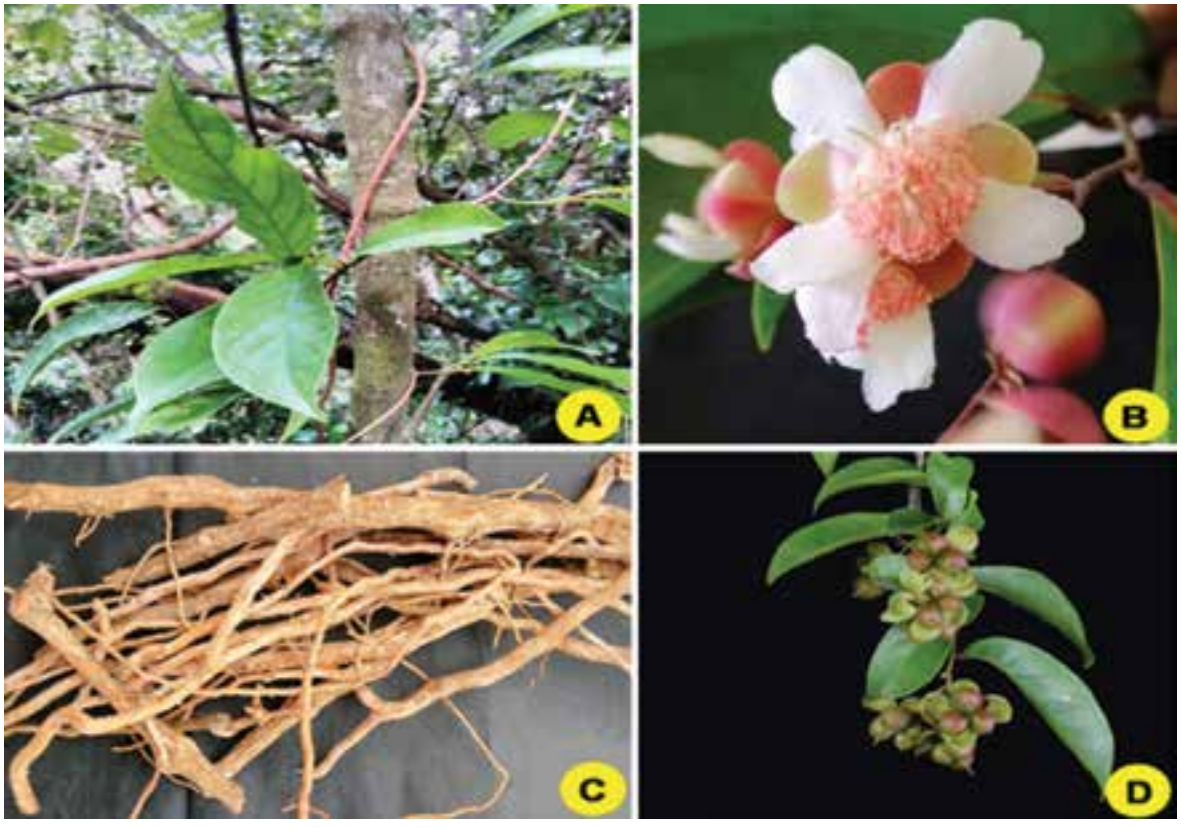

Fig.1 A. Habit of *Tetracera akara* (woody climber), B. twig with Flowers, C. roots of *T. akara* and D. twig with fruits.

2. Materials and Methods

The chemicals used during the study were of analytical grade. Solvents *viz.* petroleum ether, benzene, chloroform, acetone, ethanol (95%), n-butanol and reagents *viz.* phloroglucinol, glycerine, HCl, chloral hydrate and sodium hydroxide were procured from Sigma-Aldrich, USA. Compound microscope, glass slides, cover slips, watch glass and other common glass wares were the basic apparatus and instruments used for the study. Microphotographs were taken using a Zeiss axiostar plus microscope attached with Cannon power shot A620.

2.1 Collection and authentication of plant material

T. akara (Burm. f.) Merr. root was collected from Kottoor (N 08°35'03.8'', E 77°10' 54.8'' and altitude 585m), Thiruvananthapuram district of Kerala, India and authenticated by the plant taxonomist of the JNTBGRI, Palode. Voucher specimens were deposited at the Jawaharlal Nehru Tropical Botanic Garden and Research Institute Herbarium (TBGT 86868 dated 08/08/2015). The roots of the plant collected were washed in running water, shade dried, powdered and passed through 40 mesh sieve and stored in an airtight container for further use.

2.2 Macroscopic and organoleptic characterization

Morphological studies were carried out by observing the plant parts with naked eyes or hand lens if needed. The macroscopic and organoleptic characters like phyllotaxy, size, shape, venation, presence or absence of petiole, apex, margin, base and lamina of the leaves were noted along with texture, colour, surface, odour and taste of leaves, stem and roots (Trease and Evans, 2002 and Wallis, 1985).

2.3 Microscopic characterization

2.3.1 Anatomical studies of the Leaf, Stem and Root

Free hand transverse sections of lamina and midrib, stem and root were prepared, stained with Safranin, mounted on glass slides using glycerine and observed under light microscope with camera attachment and photomicrographs were taken (Trease & Evans, 2002).

2.3.2. Quantitative leaf microscopy

2.3.2.1. Stomatal number and stomatal index

For epidermal studies, Shultze's method of maceration was used (Subhramanyam, 1996). Leaves were first treated with conc. nitric acid for 30 min under sunlight. The upper and lower epidermis of the leaf were peeled separately and then boiled it with sodium hypochlorite solution. The peeled epidermis was placed on a slide and mounted with a drop of glycerin. An average number of stomata per mm^2 of the epidermis of the leaf (stomatal number) is calculated and the values for upper and lower epidermis were determined separately using the equation:

$$\text{Stomatal index (SI)} = \frac{S \times 100}{E+S}$$

Where, S = the number of stomata per unit area and E = the number of epidermal cells in the same unit area of leaf.

2.3.2.2. Determination of vein-islet number and vein-let termination number

The vein-islet number is the average number of vein-islets per mm^2 of a leaf surface midway between midrib and margin and the average number of terminated vein-let per mm^2 of a leaf

was taken as vein-let termination (Trease and Evans, 2002).

2.4. Powder microscopy

Fresh roots were washed under running water, shade dried, finely powdered and stored in air tight container. A small quantity of root powder was placed on slides and mounted in 2-3 drops of chloral hydrate and each slide was covered with a cover slip and then examined under a microscope. Different cell components *i.e*. cork cells, sieve tubes fibers, lignified fibers, cortex cells, calcium oxalate crystals etc. were noted and photographs were taken using a digital camera attached to a microscope (Mehlhorn, 2011).

2.5. Fluorescence analysis

The fluorescence character of the root powder (40 mesh) was studied both in daylight and UV light (254 and 366 nm) after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride etc. (Kokashi *et al*., 1958). The colour changes were noted using Methuen handbook of colour (Kornerup & Wanscher, 1978).

2.6. Physicochemical analysis

The following physicochemical parameters of *T. akara* root powder were determined according to the WHO guidelines on quality control methods for medicinal plant materials.

2.6.1. Determination of pH

Dissolved 1 g of the root powder in 100 mL and 10 g of the leaf powder in 100 mL of distilled water, filtered and checked the pH of the filtrate with a standardized glass electrode.

2.6.2. Determination of soluble extractive

Take 5 g of the air dried root powdered in 100 mL of respective solvents (hexane, chloroform, petroleum ether, ethanol, methanol and water)in a closed flask, shaked frequently during 6 h and allowed to stand for 18 h. The filtrate is collected and evaporated to dryness in a tared flat bottom shallow dish, dried at 105˚C and weighed. The percentage of soluble extractive is calculated with reference to the airdried drug.

2.6.3. Loss on Drying (LOD)

About 2-3 g of root powder is accurately weighed and kept in a hot air oven maintained at 105˚C for 5 hin a China dish. After cooling in a desiccator, the loss in weight was recorded and repeated till constant weight was obtained.

$$\text{Loss on drying (\%) LOD} = \frac{\text{Lose in weight}}{\text{Weight of the drug in g}} \times 100$$

2.6.4 Swelling Index

Leaf powder (1 g) was taken in a measuring cylinder (25 mL) and suspended in 25mL distilled water for 1 h by thorough mixing every 10 minutes. After 3 h, volume in mL occupied by the plant material including any sticky mucilage was measured. The experiment was repeated three times for accuracy and the swelling index was calculated.

2.6.5. Foaming index

Finely powdered root (1g) was boiled in 100 mL of water for 30 minutes. Then cooled and filtered into a 100 mL volumetric flask and added sufficient water to make up the volume. The prepared decoction was poured into 10 stoppered test tubes each ranging from 1 mL, 2 mL …….10 mL and the volume of the liquid in each tube was

adjusted to10 mL with water. The tubes were duly stoppered and shaken them in a lengthwise motion for 15 sec. (two shakes per second) and allowed to stand for 15 minutes. The foam height in each tube was measured.

$$\text{Foaming index} = \frac{1000}{a}$$

('a' is the volume of the plant decoction for foaming foam of height 1cm.)

2.6.6 Determination of total ash, acid insoluble ash and water-soluble ash

About 6 g root powder in a tared silica dish was ignited. Scattered the powder drug on the bottom of the dish and incinerated by gradually increasing the heat not exceeding dull red heat until free from carbon, then cooled and weighed. The % w/w of total ash with reference to the air dried drug was calculated. One part of the total ash obtained was boiled for 5-10 min with 25 mL of diluted hydrochloric acid, collected the insoluble matter in a Gooch crucible, washed with hot water, ignited and weighed. Percentage of acid insoluble ash is calculated with reference to the airdried drug. The % w/w of acid insoluble ash with reference to the air dried drug was calculated. Another part of the total ash was boiled with 25 mL of water for 5-10 min. The insoluble matter was collected in a Gooch crucible, washed with hot water and ignited in a crucible for 15 min at a temperature not exceeding 450°C. The weight of insoluble matter was substracted from the weight of the ash. The difference in weight represents the water-soluble ash. Percentage of water soluble ash is calculated with reference to the airdried drug.

2.7. Preparation of serial fractions

T. akara root powder was first extracted with ethanol to obtain crude ethanolic extract (TA CRD). Fresh root powder was then used for serial extraction with hexane using Soxhlet apparatus, the powder was then dried and again extracted with chloroform and finally with ethanol to get; 1. Hexane fraction (TA HEX), 2. Chloroform fraction (TA CHL) and 3. Ethanolic fraction (TA ETH). Yield in g/100g root powder of TA CRD, TA HEX, TA CHL and TA ETH of *T. akara* root powder was calculated. Consistency, colour and odour were also evaluated.

2.8. Phytochemical analysis

2.8.1. Preliminary phytochemical screening

Preliminary phytochemical analysis was carried out in crude, hexane, chloroform and ethanol fractions of *T. akara* using standard procedures (Kokate *et al*., 2009).

2.8.2. High Performance Thin Layer Chromatography (HPTLC) Profiling of *T. akara*

The standardization of a crude drug is an integral part of establishing its correct identity. The results of this investigation could therefore, serve as a basis for proper identification of the plant. Chromatographic fingerprint profile of hexane, chloroform, ethanol and crude extract were studied by HPTLC (CAMAG). 5 mg/ml concentration of extracts were prepared in respective solvents of chromatographic grade and then filtered by Whatman's filter paper No. 1. Prepared samples of different extracts were applied on TLC aluminum sheets silica gel 60 F 254 (Merck), 07 µl each with band length of 5 mm using Linomat 5 sample applicator set at a speed of 150 mL/sec. A number of solvent systems were tried for each extract for better resolution and maximum number of spots, but the satisfactory resolution was obtained in the

solvent system Benzene: Ethyl Acetate: Acetic Acid in the ratio 3.6 : 1.2 : 0.2. The chromatograms were developed in twin trough glass chamber saturated with solvent Benzene: Ethyl Acetate: Acetic Acid in the ratio 3.6 : 1.2 : 0.2 for 20 minutes up to the distance of 80 mm. The airdried plates were viewed under UV and day light. Spots were visible without derivatization at 254 and 366 nm wavelengths, but best results were shown when TLC plates were sprayed with detection reagent (Anisaldehyde-Sulphuric acid reagent and plate was heated at 110°C for 5 minutes) and then visualized in visible light. Scanning was performed by CAMAG HPTLC Densitometer (Scanner 3) in absorbance mode at both 254 and 366 nm, the extracts were also scanned at 350-600 nm using Deuterium and Tungsten lamp with slit dimension 6.0 X 0.45 macro. The R_f values and colour of the resolved bands were noted.

3. Results

3.1 Macroscopic and organoleptic characterization

Macroscopic and organoleptic characters of the fresh leaves, stem and root were noted and the results were presented in Table 1 and 2 and the habit of the plant is shown in Fig. 1.

Table: 2 Organoleptic characters of *Tetracera akara*

Macroscopic Observation		
Phyllotaxy		Alternate
Type		Simple
Leaf	Length	17.5 - 20.5 cm
	Width	5.00 - 6.5 cm
Shape		Elliptic-oblong
Apex		Acuminate
Margin		Entire to serrate
Venation		Reticulate
Base		Attenuate
Petiole		5 - 9 mm long.

3.2 Microscopic characterization

3.2.1 Anatomical studies of *T. akara*

Root - Cross section of root is circular in out line with outer bark, secondary cortex, phloem, peripherally placed proto xylem and meta xylem towards the pith. Parenchymatous medullary rays are present. Pith is reduced and parenchymatous (Fig. 2.A and A1).

Stem - Cross section of stem is circular in outline with outer periderm, secondary growth with peripherally placed meta xylem and proto xylem towards the center. Conjoint, open primary vascular bundles are arranged as broken ring above the parenchymatous pith (Fig. 2.B and B1).Young stem is sccabrid with multicellular trichomes.

Table: 1 Macroscopic characters of *Tetracera akara*

Organoleptic characters				
		Leaf	**Stem**	**Root**
Surface		Rough	Scabrid	Smooth
Colour	Upper	Dark green	Dark brown	Light brown
	Lower	Light green		
Odour		No characteristic odour	No characteristic odour	No characteristic odour
Taste		No characteristic taste	Slightly bitter	Bitter

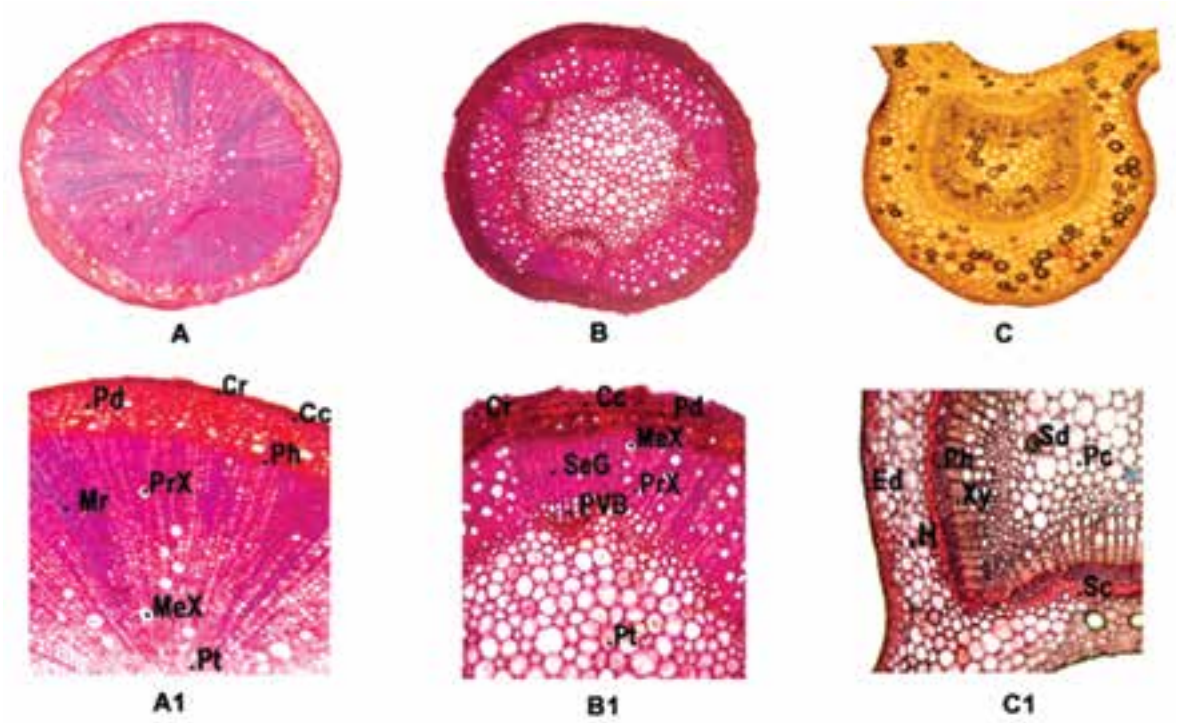

Fig: 2 A. Cross Section of root and A1. a portion of root enlarged, B. Section of Stem and B1. a portion of stem enlarged, C. Section through midrib and C1. a portion of midrib enlarged. Cr-Cork, Cc-Cork cambium, Pd-Phelloderm, Ph-Phloem, Mr-Medullary rays, PrX -Protoxylem, MeX-Metaxylem, Pt-Pith, PVB- Primary vascular bundles, SeG-Secondary growth, H-Collenchymatous hypodermis, Sc-Sclernchyma, Xy-Xylem, Sd-Starch deposition and Pc-Parenchymatous pith.

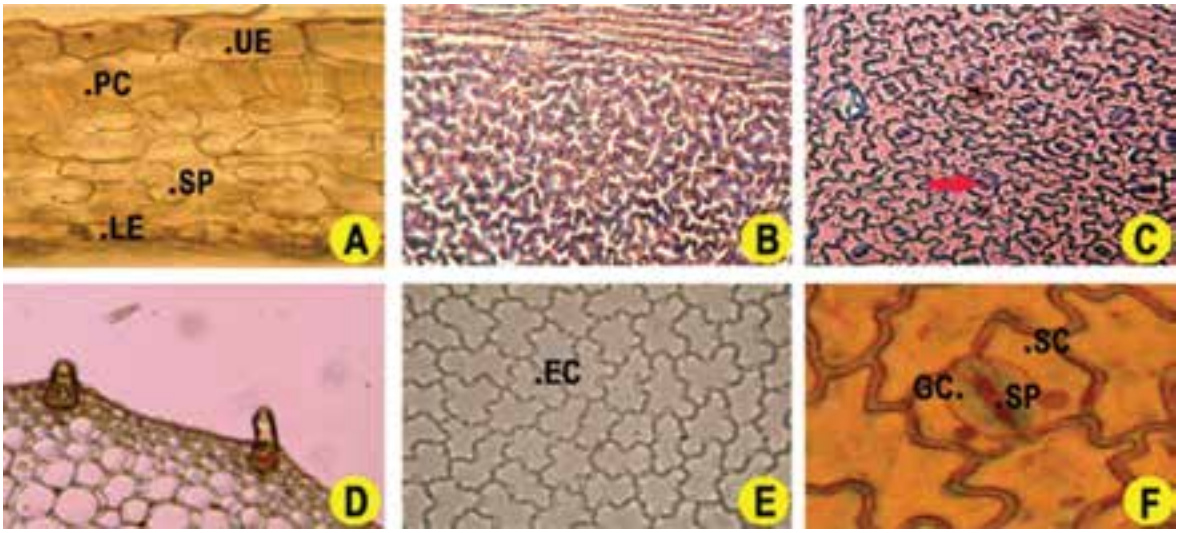

Fig. 3. A. Transverse section of lamina, B. Adaxial epidermal layer, C. Abaxial epidermal layer D. Uniseriate trichomes, E. Portion enlarged-upper epidermal layer and F. Paracytic stomatal complex. UE-Upper epidermis, PC-Palisade cells, SP-Spongy parenchyma, LE-Lower epidermis, Stomata (→) EC-Epidermal cells, SP-Stomatal pore, SC-Subsidary cell and GC-Guard cell.

Leaf - Section of the leaf shows two distinct regions, midrib and lamina.

Midrib - Single layered epidermis of rectangular cells with thin layer of cuticle. Abaxial epidermis with small rectangular cells and uniseriate trichomes with tapering end. 2-3 layers of collenchymatous hypodermis followed by 5-6 layers of parenchymatous cortex. Compactly packed parenchyma with cluster of raphide crystals are also present in cortex.1-2 layers of sclerenchyma present below the endodermis, followed by phloem and xylem. (Fig. 2.C and C1)

Lamina - Narrow lamina with single layered epidermis. The adaxial epidermis completely lacks stomata. Below the upper epidermis, compactly arranged palisade tissue is present which is followed by spongy parenchyma. Abaxial epidermis possess paracystic stomata (Fig.3).

3.2.2 Quantitative leaf microscopy

Quantitative leaf characteristics were observed and the results were shown in the Table: 3

Table: 3 *Tetracera akara* quantitative leaf microscopy

Parameter	Range	Mean ± SD
Stomatal number-Upper epidermis	0	0
Stomatal number-Lower epidermis	56 - 68	64.56 ± 5.68
Subsidiary cell length	22 - 27 µm	24.85 ± 4.2 µm
Subsidiary cell width	3 - 5 µm	4.04 ± 0.26 µm
Stomatal index-Upper epidermis	0	0
Stomatal index-Lower epidermis	15.12 – 21.28	18.67 ± 1.23
Vein islet number	19 - 26	22.8 ± 1.42
Vein let termination number	34 - 46	38.42 ± 1.82

Values are expressed as mean ± SD of ten values

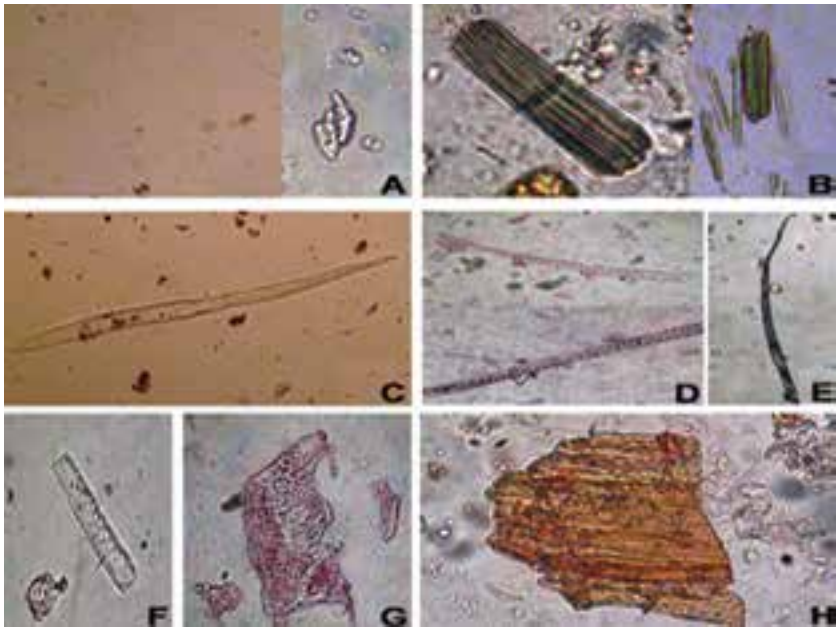

Fig. 4 Powder analysis of *Tetracera akara* shows the presence of A- Calcium oxalate crystals, B- Raphide crystals C- Xylem fibre, D - Xylem vessel, E-xylem trachieds, F- Xylem vessels with pitted thickening and G- fragments of bark.

3.3 Powder analysis

Analysis of *T. akara* root powder revealed the presence of calcium oxalate crystals (A), raphide crystals (B) xylem fibre (C), xylem vessel (D), xylem trachieds (E), Xylem vessels with pitted thickening (F) and periderm (G) shown in Fig. 4.

3.4. Fluorescence analysis

Chemical tests of *T. akara* root powder was carried out with different reagents and observed under UV- 254 nm and UV-366 nm. The results were compared with their respective observations in visible light and they were represented in Table:4 and Fig. 5.

Table: 4 Observations of *Tetracera akara* root powder under visible light and UV (254 nm and 366 nm)

Treatment	Observation (Colour developed)		
	Visible light	**UV-254nm**	**UV-366nm**
Powder alone	Orange brown (5A2)	Yellowish brown (5E8)	Dark brown (4F5)
Powder + 1 M NaOH	Brown (7E6)	Dark green (27F4)	Violet brown (10E4)
Powder + 1 M NaOH + Methanol	English red (8D8)	Dull green (26E3)	Dull green (27E4)
Powder + 1 M NaOH + Water	Dark brown (7F7)	Dark green (28F7)	Violet brown (10E3)
Powder + 1 M HCl	Orange white (5A2)	Pale green (28A3)	Pale yellow (2A3)
Powder + dil HNO_3	Butter yellow (4A5	Greenish white (27A2)*	Wax white (2B3)
Powder + 5% Iodine	Reddish brown (8E4)	Dark green (28F2)	Dark blue (19E6)
Powder + 5% $FeCl_2$	Bronze brown (5E5)	Dark green (26F5)	Blackish blue (20FC)
Powder + dil Ammonia	Orange red (8B7)	Megro (6F3)	Greyish green (29B5)
Powder + Methanol	Butter yellow (4A5)	Champagne (4B4)	Light green (28A5)
Powder + HCl	Yellowish brown (5D8)	Brass (4C7)	Greenish white (27A2)*
Powder + 1M H_2SO_4	Yellowish white (4A2)	Greenish white (28A2)	Wax white (2B3)
Powder + HNO_3	Persian orange (6A7)	Golden brown (5D7) *	Corn (4B5)
Powder + $K2Cr_2O_7$	Brownish yellow (5C8)	Topaz (5C5)	Golden blonde (5C4)
Powder + 95% Ethanol	Amber yellow (4B6)	Corn (4B5)	Mustard yellow (3B6)
Powder + Toluene	Colourless	Yellowish white (4A2)	Paster green (28A4) *

Methuen hand book of colour. *presence of fluorescence

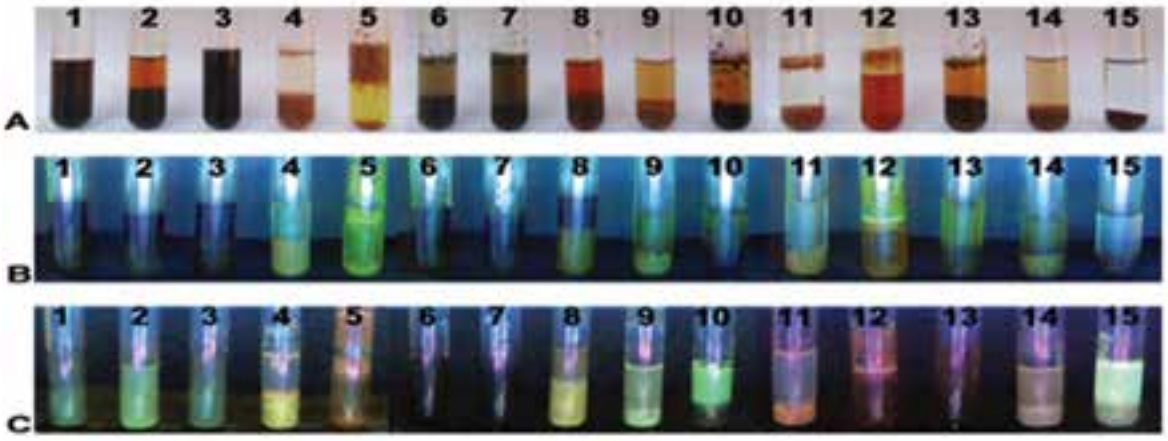

Fig. 5. *T. akara* root powder after reaction with reagents, A - Root powder observed under visible light after reaction with reagents, B - Root powder observed under UV 254 nm after reaction with reagents and showing fluorescence in 5 and 12, C -Root powder observed under UV 366 nm after reaction of with reagents and showing fluorescence in 10 and 15.

3.5. Physicochemical analysis

Physiochemical parameters of *T. akara* root powder were evaluated and the observations are presented in Table. 5.

Table: 5 Physicochemical parameters of *T. akara* root powder.

pH of Water solution	1% w/v	7.68 ± 0.42
	10% w/v	8.24 ± 0.34
Hexane-soluble extractive		0.58 ± 0.08% w/w
Chloroform-soluble extractive		1.08 ± 0.15% w/w
Petroleum ether-soluble extractive		2.78 ± 0.16% w/w
Ethanol-soluble extractive		11.26 ± 0.38% w/w
Water-soluble extractive		5.72 ± 0.35% w/w
Loss on drying (LOD)		10.8 ± 2.52% w/w
Total ash		17.46 ± 1.46% w/w
Acid-insoluble ash		1.36 ± 0.64% w/w
Water-soluble ash		14.82 ± 0.89 % w/w
Swelling index		7mL
Foaming index		250

Values are expressed as mean ± SD of six values

3.6. Extractive percentage and characteristics of extract and fractions of *T. akara* root

Physical characteristic and percentage yield of TA CRD, TA HEX, TA CHL and TA ETH were shown in the Table 6.

Table: 6 Percentage extractive and characteristics of *T. akara* root extract and fractions.

Name of the Extract/ Fractions	Consistency	Colour	Odour	Yield (g/100 g powder)
TA HEX	Semi solid	Greyish white	Characteristic	0.58 ± 0.08% w/w
TA CHL	Solid	Whitish yellow	Characteristic	1.08 ± 0.15% w/w
TA ETH	Hard solid	Dark brown	Characteristic	9.04 ± 0.19% w/w
TA CRD	Hard solid	Dark brown	Characteristic	11.26 ± 0.38% w/w

Values are expressed as mean ± SD of three values

3.7. Phytochemical analysis

Preliminary phytochemical analysis of *T. akara* crude extract and fractions of hexane, chloroform and ethanol revealed the presence of phytochemicals like flavonoids, phenols, steroids and terpenoids represented in Table. 7.

Table: 7 Preliminary phytochemical analysis of *T. akara* extracts

Phytochemicals	Test conducted	Results			
		TA HEX	TA CHL	TA ETH	TA CRD
Alkaloids	Mayer's test	-	-	-	-
	Dragendorff's test	-	-	-	-
Carbohydrates	Benedict test	+	-	+	+
	Molisch's test	-	-	+	+
Cardiac glycosides	Keller-killani test	-	+	-	+
Flavonoids	Alkaline reagent test	-	-	+	+
	Shinoda test	-	-	+	+
Phenols	Lead acetate test	-	-	+	+
	Shinoda test	-	-	+	+
Proteins	Biuret test	+	-	-	+
Saponins	Foam test	-	-	+	+
Steroids	Salkowski test	+	+	+	+
	Libermann-burchard test	+	+	-	+
Terpenoids	Solkowiski test	+	+	+	+
Tannins	Ferric chloride test	-	-	+	+
	Phenazone test	-	-	+	+

+: Present, -: Absent.

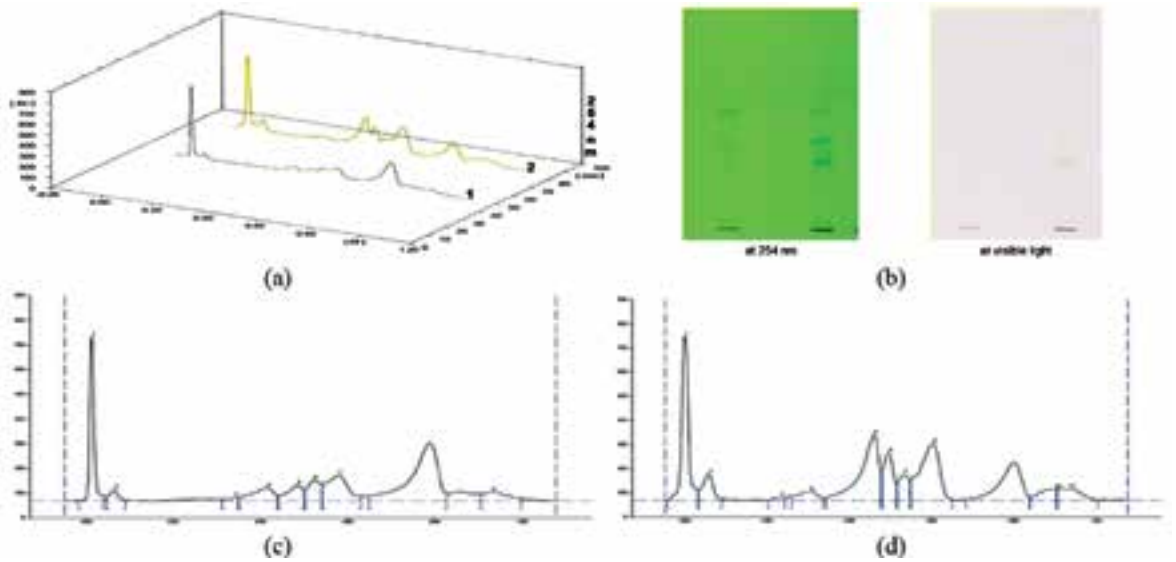

Fig: 6. a- Tracks of *T. akara* in HPTLC profiling at 254 nm, b- Chromatographic plates at 254 nm and visible light, c & d- Chromatogram of *T. akara* crude extract and Ethanol fraction at 254 nm.

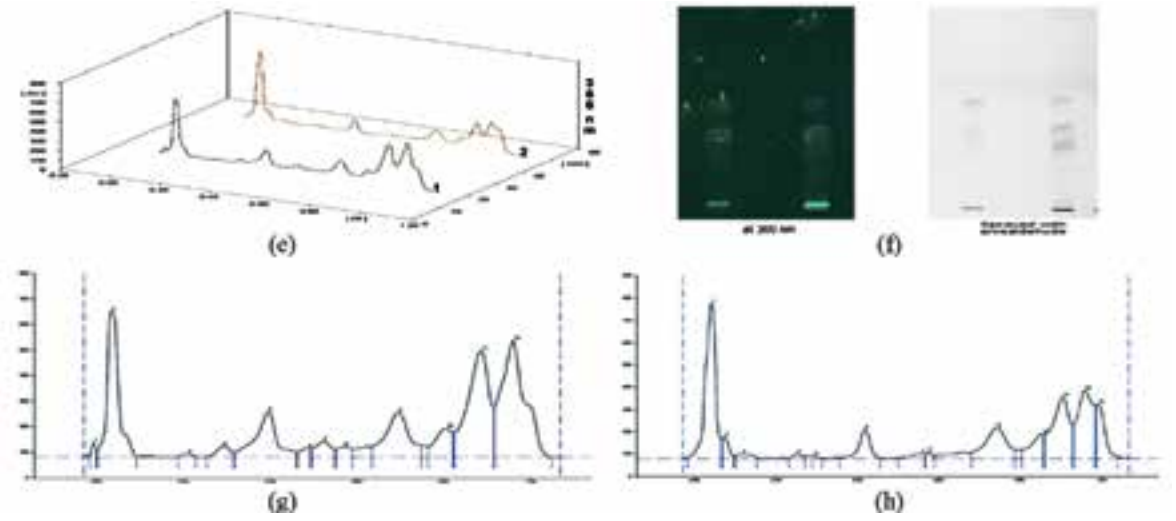

Fig: 7. e- Tracks of *T. akara* in HPTLC profiling 366 nm, f- Chromatographic plates at 366 nm and sprayed with anisaldehyde, g & h- Chromatogram of *T. akara* crude extract and Ethanol fraction at 366 nm.

3.7.1. TLC standardization and HPTLC profiling of *T. akara*

TLC of various solvent extract were carried out with many ratios of different solvents and the best eluent mixture (Benzene: Ethyl acetate: Formic acid in the ratio 3.6:1.2:0.5) was further used for HPTLC profiling. HPTLC profiling of *T. akara* crude extract and various fractions at 254 nm and 366 nm is represented in Fig. 6 & 7and the number of peaks and R_f value of phytoconstituent with % maximum is represented in Table 8.

Table: 8. Shows the number of peaks and % maximum of phytoconstituent with respective R_f value of *T. akara* crude extract and fractions at 254 nm and 366 nm.

Extract	254 nm		366 nm	
	No. of Peaks	% max with respective Rf value	No. of peaks	% max with respective Rf value
TA CRD	8	18 % at 0.79	10	21 % at .96
TA ETH	10	14 % at 0.46	11	14 % at .96

4. Discussion

Same plant may have different vernacular names in different regions of the country, which causes serious problems in identification of plants (Shinde *et al*., 2009) and can even lead to adulteration (Zhang *et al*., 2012). According to World Health Organization (WHO), the macroscopic and microscopic description of medicinal plant is the first step towards establishing its identity and purity (Anonymous., 1998). The source and quality of raw materials play an important role in guaranteeing the quality and stability of herbal preparations (Calixto, 2000). Thus, in recent years there has been an emphasis in standardization of tribal medicinal plants of therapeutic potential by pharmacognostical studies, as it is more reliable, accurate and inexpensive. Pharmacognostic characterization including physiochemical evaluation is meant for identification, authentication, detection of adulteration and compilation of quality control of the raw drug material. Since the plant, *T. akara* is used by the Kani tribes of Kerala in their traditional medicinal system, it is necessary to standardize it as the first step towards scientifically validating it as a potent source of drug against various liver ailments.

The morphological characters of *T. akara* observed can be used to distinguish it from the closely related species like *Tetracera indica*. The young stem is scrabid in nature due to the presence of large number of uniseriate trichomes and at maturity the stem become woody. Leaves are simple, elliptic to oblong in shape, which are arranged in an alternate manner. Anatomical features are important in systematics for identification, placing anomalous groups in satisfactory positions in classification and for indicating patterns of relationships that may have been observed by superficial convergence in morphological features (Essiett *et al*., 2012). The mature roots and stem of *T. akara* is woody because of secondary growth and is difficult to distinguish in powdered or chopped form. Anatomical studies of the root showed the

presence of collateral vascular bundles with endarch xylem, while stem show the presence of conjoint vascular bundles with exarch xylem. Large parenchymatous pith is present in the C.S of stem while pith is highly reduced in roots. The anatomical features described above can be used to identify the correct raw material, the root with higher phytochemical content. Transverse section of leaf shows midrib and laminar regions with single layer of epidermis. Adaxial surface of the leaf completely lack stomata and paracystic stomata are found on the abaxial surface with a stomatal index of 18.67 ± 1.23. Presence of uniseriate trichrome on the lower side of leaf is noted.

Powder microscopic analysis showed the presence of calcium oxalate crystals, raphide crystals, xylem fibre, xylem vessel, xylem trachieds, xylem vessels with pitted thickening and fragments of bark. When physicochemical methods become inadequate, the plant materials can be distinguished from their adulterants on the basis of fluorescence characterization. *T. akara* root powder produced characteristic fluorescence in short UV when treated with dilute and concentrated HNO_3 and in long UV when treated with HCl and Toluene. Various physiochemical parameters evaluated in this study can be used for adulterant resolution or improper handling of the raw material and compilation of a suitable monograph for *T. akara.* Low moisture content indicates less chances of microbial degradation of plant drug during storage (Kunle *et al*., 2012). The general requirement of moisture content in herbal drugs is less than 14 % (Anonymous., 1980). In this study, the loss on drying indicate the moisture content which is 10.8 ± 2.52 % w/w and within the accepted range.

Ash values can be used as reliable aid for identification of the plant materials and detecting adulteration (Nayak *et al*., 2010). It gives an idea of earthy matter or the inorganic composition and other impurities present along with drug. Based on the result obtained, the total ash value obtained was 17.46 ± 1.46 % w/w, acid-insoluble ash was 1.36 ± 0.64 % w/w and water soluble ash was 14.82 ± 0.89 % w/w respectively. The acid insoluble ash was very low which shows that a very small amount of the inorganic component is present which is insoluble in acid and this is of diagnostic importance. The extract values give an idea about the nature of the chemical constituents present in the plant and is useful for the estimation of specific constituents soluble in that particular solvent. The ethanol and water soluble extractive yield were higher than that of hexane, chloroform and petroleum ether fractions. Thus, ethanol and water are good choice of solvent extraction of *T. akara*.

Phytochemical analysis is one of the important tool for quality assessment of medicinal plant which includes preliminary phytochemical analysis, chemo profiling and marker compound analysis using modern analytical techniques. Comparative preliminary phytochemical analysis revealed the phytochemical nature of various solvent extract of *T. akara* root, of which the crude extract and ethanolic fraction were rich in bioactive phytoconstituents like flavonoids, phenols, tannins, saponins and terpenoids. Flavonoids and phenols possess cardioprotective, lipid lowering, antiulcer, hepatoprotective, anti-inflammatory, antineoplastic, antibacterial, antifungal, anti-allergic, antiviral and antioxidant properties (Gupta., 2010). The tannins possess antimicrobial, anti-oxidant and antihypertensive

properties (Salas *et al*., 2010). Anti-cancerous effect of saponins has been reported earlier (Francis *et al*., 2002). Triterpenoids possess wound healing, anti-inflammatory, antiviral and hepatoprotective effects (Jäger *et al*., 2009).

TLC standardization of various solvent extract of *T. akara* with different ratios of solvents showed that maximum separations of the phytoconstituents were obtained in the solvent system Benzene: Ethyl acetate: Formic acid in the ratio 3.6:1.2: 0.5. This solvent system was further used for HPTLC profiling. HPTLC method has been used as a reliable tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations (Sajeeth *et al*., 2010). HPTLC fingerprint profiling of crude and ethanolic fraction of *T. akara* were carried out for the first time and it will serve as a reference standard in medicinal plant research. Maximum number of peaks at 254 nm were obtained for *T. akara* ethanolic fraction. At 366 nm, a maximum of 11 peaks were obtained for *T. akara* ethanol fractions. This analysis was the first of its kind towards understanding the nature of active principle and detailed phytochemistry of *T. akara*. However, isolation of individual phytochemical constituents from *T. akara* will pave way for the development of a novel herbal drug with least side effects.

5. Conclusion

The present study was undertaken with an aim of pharmacognostic standardisation and phytochemical evaluation of *T. akara* root which is used by the Kani tribe of Kerala to treat various liver ailments. These parameters which are being reported for the first time in this study will help in authenticating the genuine plant material. It will also help in detecting adulterant and setting some diagnostic indices for the identification and preparation of monograph of *T. akara*.

Acknowledgements

The authors would like to thank Department of Science and Technology, India, INSPIRE Programme for financial assistance and Director, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, for the providing necessary facilities.

Conflict of Interest

The authors have no conflict of interest.

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