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Traditional method of preparation and development of quality standards for an edible vegetable fat Malabar tallow (*Vateria indica* L. seed butter)

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Dhupa fat or Malabar tallow is an edible fat extracted out of *Vateria indica* L. seed and is a traditional source of edible fat in the household of Malabar region. This investigation is an attempt to record macro-microscopic features of *V. indica* L. fruits along with physico-chemical characterization of seed and butter. The methodology of traditional extraction, analytical standardization and chemical fingerprints of the seed butter have been documented following standard procedure. Fragments of xylem, oil cells, sclerenchyma fibres are features of powder microscopy. Glycosides and coumarins were said to be main secondary metabolites detected out of the test drug. Analytical specifications of *V. indica* L. butter has shown saponification value and iodine value as 112.43 and 8.82 respectively. HPTLC photo documentation of chloroform and ethanolic extract of test drug given 1 and seven spots at 366 nm respectively. Thus current study derives prime scientific data on *V. indica* fruit, seed butter and their chemical nature.

Keywords: Dhupa, Edible vegetable fat, Natural product resource, Pharmacognosy, Standardization.

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Introduction

Vateria indica L. known commonly as Indian copal tree or white dammar is a large, elegant, evergreen tree endemic to evergreen forests of Western Ghats[1]. It is also extensively planted as an avenue tree in Karnataka. The resin exuded by the tree is known as piney resin, white dammar or Dhupa and is commercially important as an aromatic drug[2]. Apart from this, the tree yields an abundant quantity of fruits which are used for preparing an edible fat[3]. The fruit comprises a thick pericarp with semi fibrous composition, weighing about a half portion of the whole fruit. On an average, a fully matured tree yields about 400 to 500 kg of fruits. Seeds are hard and brittle and have a faint aromatic odour. These yield a semi-solid fat known as piney tallow, Malabar tallow or Dhupa fat. It is used for the edible purpose, confectionery and as an adulterant of ghee[4]. It has been suggested for use in blends with cocoa-butter or as its substitute. Local people of Dakshina Kannada use butter extracted from seed for cooking dosa, papad and other dishes[1,2]. In addition to this therapeutically it is used as a local application in rheumatism and allied afflictions[5].

In India, a great deal of bulk knowledge exists among ordinary people about the traditional use of herbal medicine. Global herbal supplements and the medicinal market is portended to reach $107 bn by 2017 by rising ageing inhabitants and consumer awareness[6]. An innovative research effort to define the advantage of the traditional system of medicine with respect to their safety and efficacy could result in a better utilization of these complementary systems of medicine. Internationally several pharmacopoeias have provided monographs stating parameter and standard of many herbs and some product made out of those herbs[7].

Hence a sincere effort has been made in this paper to record detailed pharmacognostical characteristics of *V. indica* L. fruits detailing macro-microscopic and physico-chemical standards of fruit, preparation methodology of seed butter, its analytical specification and HPTLC fingerprint profiles.

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Materials and Methods

Collection and identification

V. indica seeds were collected from Udupi district of Karnataka, India during the month of June 2013. A museum specimen was deposited at SDM Centre of Research and Allied Sciences, Udupi (Voucher no. 286/13073003-04). Other physical impurities were removed from fruits, thoroughly washed with tap water and shade dried. Macroscopic features were documented using Canon Ixus digital camera. Remaining fruits are shade dried properly and used for further study[8].

Powder microscopy

A pinch of dried seed powder was mounted on a microscopic slide with a drop of glycerin-water. Characters were observed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the pre-calibrated scale-bars using Zeiss Axio Vision software[9].

Physico-chemical standardization

Procedures recommended in the World Health Organization (WHO) guidelines were followed to calculate the parameters like loss on drying, total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive[10].

Preliminary phytochemical study

Preliminary phytochemical investigations were conducted on the seed powder to find different phytoconstituents present in aqueous and alcoholic extracts[11].

Preparation of seed butter

Seed butter was prepared at *Rasashastra* and *Bhaisajyakalpana* Department, SDM College of Ayurveda, Udupi according to traditional methodology[12]. Seeds of *V. indica* L. were made into a thick paste using grinding machine, the paste was poured into boiling water in a copper vessel and stirred constantly with a spatula. Heated over a low flame to make the butter globules float over the surface of the water. These fat materials were transferred into another vessel and re-heated until the water content was removed. Fat, free from water was collected and stored in an airtight container until further study[12].

Analytical studies

V. indica L. seed butter was evaluated for analytical constants like refractive index, weight per mL**,** saponification value, iodine value, acid value, and determination of unsaponifiable matter[13].

High-performance thin layer chromatography

HPTLC fingerprinting of chloroform and ethanolic extract of *V. indica* L. seed powder was obtained as per standard procedure. About 5 and 10 µL of the extracts were applied on a precoated silica gel F254 on aluminium plates to a bandwidth of 8 mm using Linomat 5TLC applicator[14]. The plate was developed in benzene: ethyl acetate: formic acid (19:1:0.1) for chloroform extract and chloroform: methanol (7:3) for ethanol extract. Developed plates were visualized and scanned under UV (254 and 366 nm) and after derivatisation in vanillin-sulphuric acid spray reagent.

Results

Fruits are ovoid, rough, pale brown, three-valved capsules and one seeded. Seeds are reddish-white or cream coloured (Plate 1). Microscopically seed powder showed pigment cells, oil cells, fragment of mesophyll from cotyledon, epidermal cells of cotyledon, fibres, vessels and rosette crystals (Plate 2). Physico-chemical constants and preliminary phytochemical constituents of *V. indica* are presented in Table 1 and 2 respectively. Coumarins and carbohydrates were found to be main secondary metabolites present in *V. indica* L. Analytical specification of *V. indica* butter is shown in Table 3. The HPTLC fingerprint of *V. indica* L. is depicted in Fig. 1 to 4, Table 4 and 5. HPTLC fingerprint profile of the test drug was done using both chloroform and ethanolic extract. R_f, the colour of the spots, and densitometric scan were recorded. HPTLC photodocumentation of the chloroform extract of

Plate 1 — Macroscopic view of *Vateria indica* L. fruit

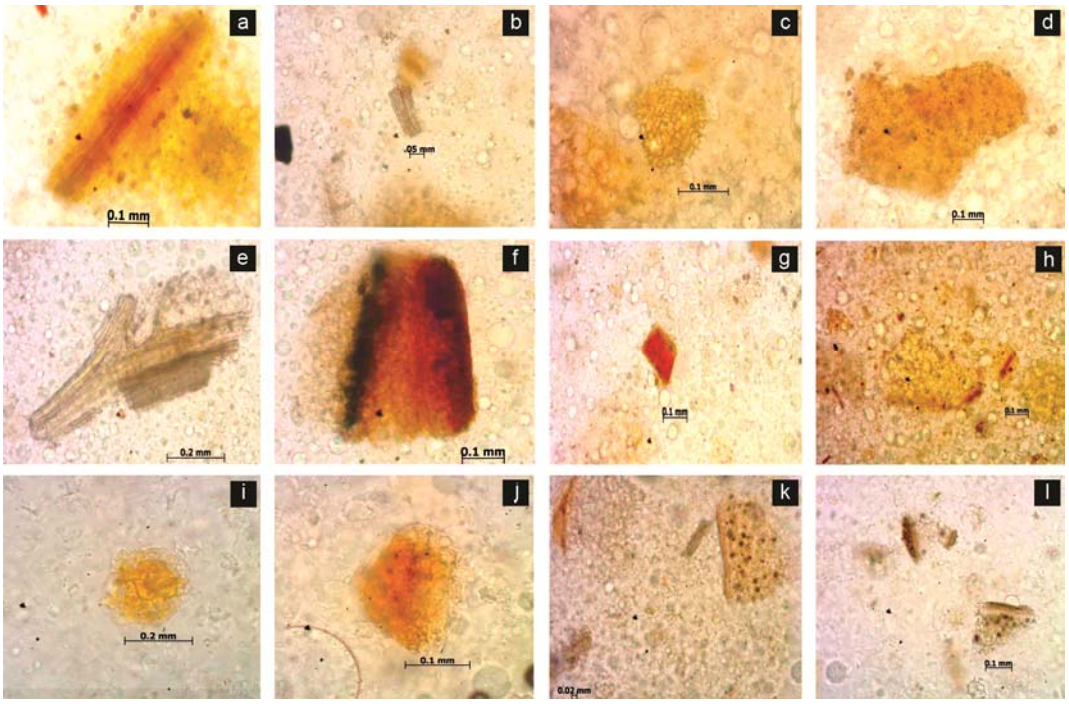


Plate 2 — Microscopy of powder of *Vateria indica* L. seed. a) Fragment of xylem with rosette idioblasts, b) Tracheidal fibres, c) Mesophyll of cotyledon, d) Epidermal cells of cotyledon, e) Fibre bundle, f) Parenchyma of cotyledon, g) Pigment cell, h) Vessel fragments, i & j) Cells with oil and colored content, k & l) Cotyledon cells with rosette crystals.

Table 1 — Physico-chemical standards of the *Vateria indica* L. seed

Tests	(% w/w) (n=3)
LOD	2.62±0.35
Total ash	3.15±0.12
Acid insoluble ash	0.90±0.02
Water soluble extractive	29.09±1.21
Alcohol soluble extractive	26.11±2.35

Table 2 — Phytochemical moieties of the *Vateria indica* L. seed

Alkaloid	-
Amino acids	-
Coumarin	+
Flavanoid	-
Carbohydrate/ glycoside	+
Steroid	-
Phenol	-
Tannins	-
Triterpenoid	-
Resins/ wax	-
Saponins	-
Quinone	-

Table 3 — Analytical specifications of *Vateria indica* L. seed butter

Refractive index	1.46544
Weight per mL	0.8992
Saponification value	112.43
Iodine value	8.82
Acid value	0.6
Unsaponifiable matter	1.88

Table 4 — R_f value of chloroform extract of *Vateria indica* seed butter

At 254 nm	At 366 nm	Post derivatisation
-	-	0.10 (Light violet)
-	-	0.18 (Light violet)
-	-	0.23 (Light violet)
-	0.25 (F Light violet)	-
-	-	0.33 (Light violet)
-	-	0.41 (Pink)
-	-	0.53 (Light violet)
-	-	0.62 (Light violet)
-	-	0.77 (Light violet)

Table 5 — R_f values of all the samples ethanol extract of *Vateria indica* seed butter

At 254 nm	At 366 nm	Post derivatisation
-	0.15 (Violet)	0.15 (Grey)
0.18 (Light green)	-	-
-	-	0.20 (Light brown)
0.2 6(Light green)	-	-
-	0.28 (Violet)	-
-	0.40 (Violet)	-
-	0.54 (Violet)	-
-	0.68 (Violet)	-
-	0.80 (Dark violet)	-
0.85 (Dark green)	-	0.85 (Light pink)
-	0.90 (Dark violet)	0.90 (Dark pink)

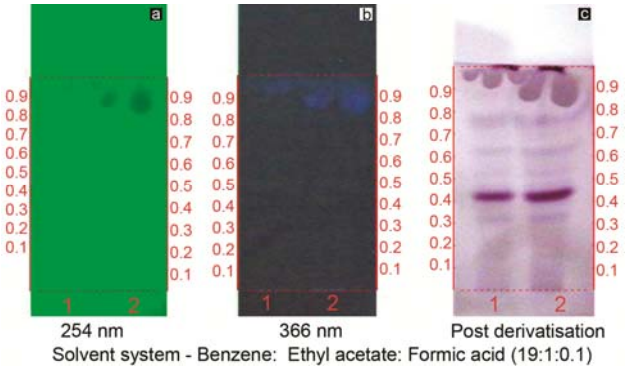


Fig. 1 — Photodocumentation of chloroform extract of *Vateria indica* seed. a) Short UV, b) Long UV, c) After derivatisation.

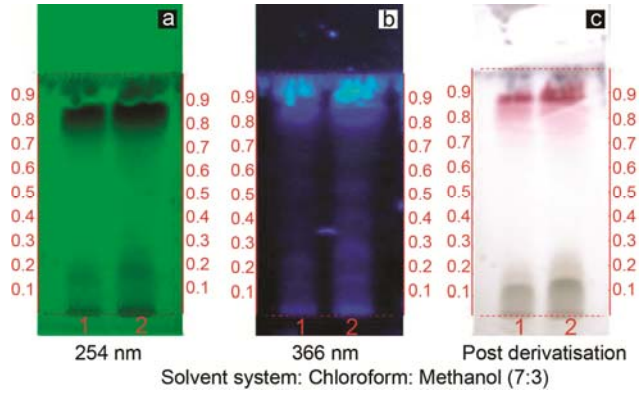
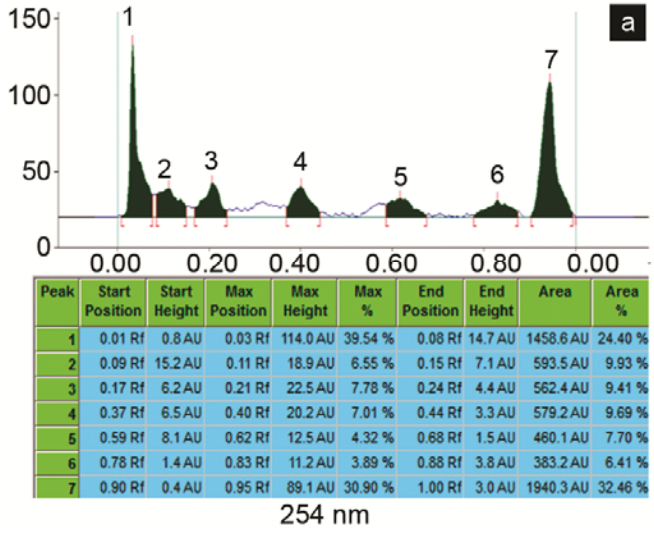
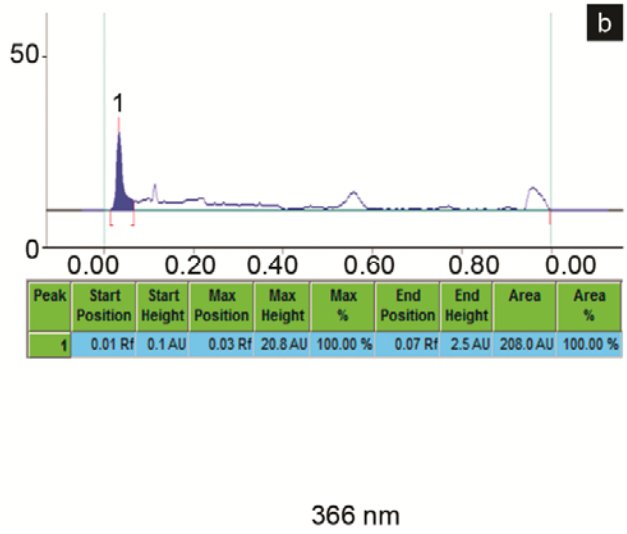


Fig. 2 — Photodocumentation of ethanol extract of *Vateria indica* seed. a) Short UV, b) Long UV, c) After derivatisation.

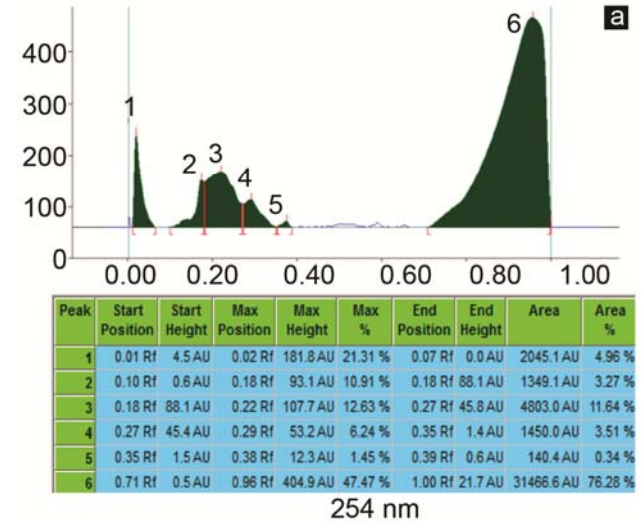


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.8 AU	0.03 Rf	114.0 AU	39.54 %	0.08 Rf	14.7 AU	1458.6 AU	24.40 %
2	0.09 Rf	15.2 AU	0.11 Rf	18.9 AU	6.55 %	0.15 Rf	7.1 AU	593.5 AU	9.93 %
3	0.17 Rf	6.2 AU	0.21 Rf	22.5 AU	7.78 %	0.24 Rf	4.4 AU	562.4 AU	9.41 %
4	0.37 Rf	6.5 AU	0.40 Rf	20.2 AU	7.01 %	0.44 Rf	3.3 AU	579.2 AU	9.69 %
5	0.59 Rf	8.1 AU	0.62 Rf	12.5 AU	4.32 %	0.68 Rf	1.5 AU	460.1 AU	7.70 %
6	0.78 Rf	1.4 AU	0.83 Rf	11.2 AU	3.89 %	0.88 Rf	3.8 AU	383.2 AU	6.41 %
7	0.90 Rf	0.4 AU	0.95 Rf	89.1 AU	30.90 %	1.00 Rf	3.0 AU	1940.3 AU	32.46 %

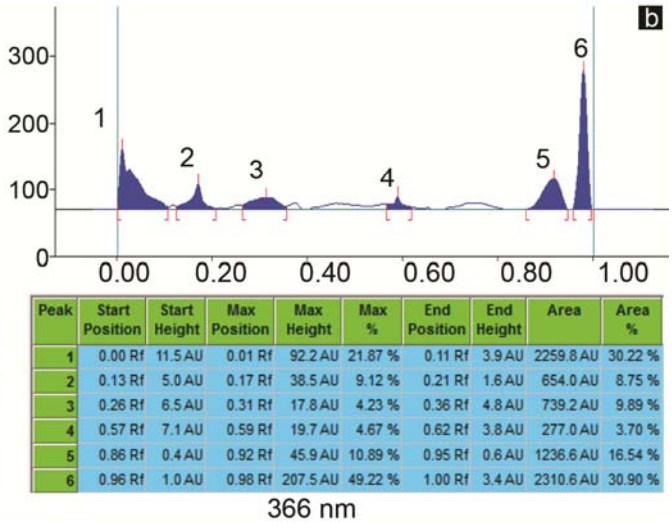


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.1 AU	0.03 Rf	20.8 AU	100.00 %	0.07 Rf	2.5 AU	208.0 AU	100.00 %

Fig. 3 — Densitometric scan of chloroform extract of *Vateria indica* seed. a) 254 nm, b) 366 nm.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.5 AU	0.02 Rf	181.8 AU	21.31 %	0.07 Rf	0.0 AU	2045.1 AU	4.96 %
2	0.10 Rf	0.6 AU	0.18 Rf	93.1 AU	10.91 %	0.18 Rf	88.1 AU	1349.1 AU	3.27 %
3	0.18 Rf	88.1 AU	0.22 Rf	107.7 AU	12.63 %	0.27 Rf	45.8 AU	4803.0 AU	11.64 %
4	0.27 Rf	45.4 AU	0.29 Rf	53.2 AU	6.24 %	0.35 Rf	1.4 AU	1450.0 AU	3.51 %
5	0.35 Rf	1.5 AU	0.38 Rf	12.3 AU	1.45 %	0.39 Rf	0.6 AU	140.4 AU	0.34 %
6	0.71 Rf	0.5 AU	0.96 Rf	404.9 AU	47.47 %	1.00 Rf	21.7 AU	31466.6 AU	76.28 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	11.5 AU	0.01 Rf	92.2 AU	21.87 %	0.11 Rf	3.9 AU	2259.8 AU	30.22 %
2	0.13 Rf	5.0 AU	0.17 Rf	38.5 AU	9.12 %	0.21 Rf	1.6 AU	654.0 AU	8.75 %
3	0.26 Rf	6.5 AU	0.31 Rf	17.8 AU	4.23 %	0.36 Rf	4.8 AU	739.2 AU	9.89 %
4	0.57 Rf	7.1 AU	0.59 Rf	19.7 AU	4.67 %	0.62 Rf	3.8 AU	277.0 AU	3.70 %
5	0.86 Rf	0.4 AU	0.92 Rf	45.9 AU	10.89 %	0.95 Rf	0.6 AU	1236.6 AU	16.54 %
6	0.96 Rf	1.0 AU	0.98 Rf	207.5 AU	49.22 %	1.00 Rf	3.4 AU	2310.6 AU	30.90 %

Fig. 4 — Densitometric scan of ethanol extract of *Vateria indica* seed. a) 254 nm, b) 366 nm.

V. indica butter has shown no spots at 254 nm, whereas 1 spot at 366 nm. Post derivatisation of the same has shown two prominent spots at R_f 0.62 and 0.77. Similarly, HPTLC photodocumentation of ethanolic extract of test drug has shown 3 spots at 254 nm, whereas at 366 nm it has shown 7 spots. Post derivatisation revealed two prominent spots at R_f 0.85 and 0.90.

Discussion

Use of medicinal herbs in the form of food is a popular practice for quality life prescribed in ancient texts of Ayurveda[15]. *V. indica* butter is greenish yellow to white, fairly soft, having a slight pleasant odour and is easily bleached on exposure to light. In the traditional method of extracting the fat, the seeds are crushed and boiled in water till the melted fat rises to the surface. It is a common source of edible fat in the household of Malabar region. The fresh fruits are reddish white and the average weight of kernel is 55 g. The fruits quickly germinate in the rainy season and need a facility for quick collection, transportation, decortication, drying and storage. The fat in the dried seeds generally varies from 22 to 27 %. In the traditional method of fat extraction, the seeds are crushed and boiled in water till the melted fat rises to the surface.

Powder microscopy plays an important role in the pharmacognostical evaluation and sometimes may be an identifying parameter of herbal drugs[16]. Fragments of xylem, oil cells, cotyledon cells with rosette crystals, sclerenchyma fibres and pigment cells are distinguishing microscopic features.

Results obtained for physico-chemical parameters such as loss on drying, pH, water soluble extractive, total ash, acid insoluble ash forms as standard values in drug identification[17]. Loss on drying (LOD) indicates the moisture content of the drug. Matured fallen fruits collected and involved in this study, have shown their value as 2.62 % (w/w). Total ash indicates the presence of inorganic salts. Minimum ash value (3.15 % w/w) shows their purity without any contamination/ adulterants. Acid insoluble ash is the residue obtained after boiling the ash with hydrochloric acid and igniting the insoluble portion. It gives a measure of sand and other silicaceous matter. Acid insoluble ash of *V. indica* was calculated to be 0.90 % w/w, which indicates less contamination with earthy matters. The solubility of *V. indica* powder material in water and alcohol was found to be 29.09 and 26.11 % w/w, respectively. Water yielded a higher percentage of extract than alcohol.

Plants are of great medicinal value as they are rich in secondary metabolites like alkaloids, glycosides, flavanoids, steroids, related active metabolites and have been extensively used as drugs in pharmaceutical industry[18]. Phytochemical analysis of *V. indica* showed the presence of glycosides and coumarins as main functional groups. Alkaloids, amino acids, flavanoids, steroids, phenols, tannins, triterpenoids, saponins, resins/wax, quinone found negative.

Seed butter obtained was brownish yellow in colour and the consistency was semisolid. Analytical specification of this has revealed its comprehensive chemical characteristics. Refractive index indicates the density of sample compared to air and liquid media; the value for *V. indica* butter was found to be 1.47583. The amount of alkali needed to saponify a given quantity of oil/ fat will depend upon a number of COOH group present; the saponification value also indicates the average molecular weight/ chain length of all fatty acids present[19]. Longer chain fatty acids have low saponification value, and the shorter chain fatty acids have high saponification value. Shorter chain fatty acids (high saponification value) have a faster rate of absorption than longer chain fatty acids; saponification value of the sample was found to be 112.43 %. Unsaponifiable matter indicates components oil/ fats other than fatty acids, the value for this sample was found to be 1.88 %. The acid value indicates the presence of free fatty acids in the oil/ fat which are responsible for rancidity of the compounds; higher the free fatty acid more is the rancidity. This helps to decide the shelf life of the oil/ fat; acid value for *V. indica* butter was found to be 6.02. Iodine value indicates the degree of unsaturation of oil/ fat; greater the degree of unsaturation, higher will be the possibility of absorption and atmospheric oxidation leading to rancidity[20]. More the iodine number, the more unsaturated fatty acid bonds are present. Unsaturated fatty acid is better absorbed than saturated fatty acids, the value for *V. indica* was found to be 8.82 (Table 2). These constants can be used as standard analytical specifications for *V. indica* butter.

HPTLC is one of the standard chromatographic techniques used for routine chemical fingerprinting of herbal drugs. It is easy and cost effective to identify botanicals using this technique. The spots and peaks obtained in *V. indica* seed would be helpful in authentication even in powder or extract form[21].

Conclusion

Traditional knowledge about plants has become the treasure trove and cultural heritage of many nations. Malabar tallow (*Vateria indica* L. seed butter) is a common edible fat extracted out of falling seeds which is abundantly available during June and July. A well-established quality control and identification parameters provided in this paper may prove as a reference standard in future research. This is a necessary step to preserve and protect traditional knowledge and also establish a database of traditional medicine.

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