

**MORPHOLOGICAL, ANATOMICAL, TOTAL SAPONIN
CONTENT AND PHYSICOCHEMICAL EVALUATION OF
SAPINDUS TRIFOLIATUS L.**

S. MUTHUPETCHI, G. ADAIKALA RAJ[#], M. ARUN SUBASH

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Department of Rural Development Science, "Arul Anandar" College (Autonomous),
Karumathur 625514, Madurai, Tamil Nadu, India, [#]e-mail: adaikalamvsp@gmail.com, Mobile:
+91 9003360322

Abstract. *Sapindus trifoliatus* Linn belongs to family *Sapindaceae* is commonly known as reetha. It is a deciduous tree with medium to large size; some species exists as shrub too. This tree is an important forest tree species. *Sapindus trifoliatus* is commonly known as soapberries and soapnuts since the fruits are used to make soap and acting as natural detergent. In the present paper was studied fruit morphology, anatomical and saponin content of the fruit of *Sapindus trifoliatus*. Morphological characterization revealed that fruit characters viz., color, shape and size with wrinkled surface and globose shape in *S. trifoliatus* and dark brown color. In particular, saponin contents in *S. trifoliatus* fruits were 0.676 µg/mL. This study contributes to a better understanding of raw or purified extract and isolated saponins are valuable plant products that can be used in the food, pharmaceutical, cosmetic, and chemical industries.

Key words: *Sapindus trifoliatus*, HPLC, fruit morphology, saponin, anatomy.

INTRODUCTION

Secondary plant metabolites provide a diverse range of substances that exhibit biological activity [1]. Modern economic advancement particularly highlights the importance of eco-friendly practices, which includes a preference for technological solutions that utilize natural, renewable materials, especially those derived from

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plants [13, 15, 16]. Detergents represent a significant category of industrial products that are meant for widespread use and have a considerable effect on the environment [5]. The use of surfactants is not limited to cleaning products. Because of their amphiphilic characteristics, which enable adsorption, emulsification, washing, or foaming abilities, these compounds have extensive applications in various industries [9, 27]. For instance, detergents function as products or additives in sectors such as food, cosmetics, pharmaceuticals, textiles and leather, as well as metallurgical and petrochemical industries [26].

Saponins are naturally occurring secondary metabolites in plants that possess surfactant characteristics [12] and are produced by both plants and certain marine organisms [24]. Chemically, they are categorized as glycosides. The term saponins comes from their resemblance to soap properties [25] with the Latin term *sapo*, translating to 'soap' [19]. When dissolved in water, saponins lower the surface tension and exhibit foaming capabilities [21]. The detergent-like properties of saponins stem from their amphiphilic nature [29], which features a hydrophobic core called a glycone [7, 20] (or genin) along with hydrophilic sugar components (glycone). The structural variation of saponins in nature is primarily based on the two glycoside-forming components. The glycone portion is made up of one or more sugar chains [30], which are linked to the aglycone through a glycosidic bond [32]. The O-glycosidic bond serves as a boundary that divides the two structural components of saponins. Saponins are mostly categorized according to differences in the structure of the aglycone or the quantity of sugar chains [8]. The primary classification based on skeletal structure identifies two key categories: steroids and triterpenoids. Steroid glycoalkaloids are occasionally categorized alongside saponins. Typically, steroidal aglycones have a skeleton made up of 27 carbon units, while triterpenoid aglycones usually contain 30 carbon units [11]. Besides variations in carbon count, the diversity in the structure of the aglycone is attributed to different types and arrangements of substituents, along with additional modifications in the backbone [36]. Saponins appear in many vascular plant families as secondary metabolites. This group also includes members of the *Sapindaceae* family, which produce saponins of the triterpenoid type [34].

Sapindus trifoliatus L. (*Sapindaceae*) is a medium to large deciduous tree primarily found in the evergreen and moist forests of southern and western India. It is commonly referred to as the "Soapnut of South India". The tree is also cultivated in various other Indian states, including Orissa, Madhya Pradesh, Uttar Pradesh, and West Bengal [28]. It grows best in loamy, clayey, and black-cotton soils, and holds significant economic value in the saponin industry. The fruits are rich in saponin, a natural detergent. Detergents are commonly used as an alternative to soap for both laundering clothes and washing hair. The root bark contains saponin and is utilized as a cleaning agent [17]. The fruit's pericarp is known for its medicinal effects, such

as emetic, tonic, astringent, and antihelminthic properties, and is employed in treating conditions like asthma, colic from indigestion, diarrhea, cholera, tubercular glands, paralysis of limbs, and lumbago. A thick, watery mixture made from the pericarp is used to alleviate hemicrania, hysteria, or epilepsy [17]. Recently, the aqueous extract of the pericarp has been noted for its pain-relieving properties [3]. Given these diverse applications, *S. trifoliatus* is experiencing significant pressure from human activities. It is, therefore, crucial to investigate the morphological, anatomical, and saponin content of the fruit of *S. trifoliatus* for its conservation and sustainable use.

METHODOLOGY

SAMPLE COLLECTION

The research material of *S. trifoliatus* was collected from Idukki district. The fruit were collected at the turn of 16th January 2023, complete ripeness.

PLANT MATERIAL

A total of 100 fully matured dry fruits were cleaned to remove any dust particles, then allowed to air dry at room temperature. At this stage, the fruits were fully ripe and displayed a consistent brown or dark brown pulp color, which varied according to genotype. Ripe fruits were selected for the evaluation of morphological traits. The fruits were carefully analyzed for qualitative attributes such as type, shape, apex, surface, base, and color, along with quantitative measures including fruit length, fruit width, peel thickness, seed length, and seed width, all assessed using a Vernier caliper. The weights of the fruit, peel, pulp, and seeds were measured with an analytical balance.

FRUIT ANATOMY

The anatomical analysis of 5–10 fruit samples (which could be schizocarps or individual mericarps) from each species were immersed in distilled water for 10 minutes. Following this, a dehydration process was carried out using a sequence of graded ethanol concentrations (80 % and 90 % for 2 hours each) and the samples were left in absolute ethanol overnight. Manual transverse slices of the pericarps were made. The samples were then viewed under a light microscope [14].

SAMPLE PREPARATION

The soapnut seed was separated from its pericarp through crushing, followed by drying the pericarp in a hot air oven for four hours to remove moisture. The dried material was then ground into a powder at a speed of 50 rpm. After this, the powdered sample was dried again and sieved to remove any impurities from the mixture. Eventually, the powder samples underwent maceration with methanol to create the soapnut extract. This extract was filtered using Whatman No. 1 filter paper placed in a glass funnel. The resulting crude extract was kept at $-20\text{ }^{\circ}\text{C}$ until required. Later, the extract was evaporated to dryness at $65\text{ }^{\circ}\text{C}$ using a hot plate method and then re-dissolved in 2 mL of methanol to prepare for the HPLC analysis.

QUANTIFICATION OF SAPONINS BY HPLC METHOD

Quantitative assessments were performed by analyzing the peak area from the chromatograms of both standards and samples. A refined method previously outlined [22] was employed for the analysis of saponins. Saponin standards were sourced from G.S. Scientific Services located in Madurai. The concentrations of the saponin A stock standard solution were, respectively, 1 mg in 1 mL of methanol, for HPLC analysis to establish their corresponding calibration curves. 0.2 to 1.0 mg/mL calibration curves were generated by plotting the logarithm of the HPLC peak areas against the logarithm of the standard concentrations. The HPLC method was carried out using a Shimadzu LC-10 AT VP HPLC system, featuring an LC-10AT pump, UV-VIS detector SPD-10AT, and a Rheodyne injector fitted with a 20 μL loop along with an auto injector SIL-10AT. A Hypersil® BDS C-18 column ($4.6 \times 250\text{ mm}$, 5 μm) accompanied by a C-18 guard column was utilized. An isocratic HPLC (Shimadzu HPLC Class VP series) employed two LC-10 AT VP pumps (Shimadzu), a variable wavelength programmable photo diode array detector SPD-M10A VP (Shimadzu), a CTO-10AS VP column oven (Shimadzu), a SCL-10A VP system controller (Shimadzu), and a reverse phase Luna 5 $^{\circ}\text{C}$ 18 Phenomenex column ($250\text{ mm} \times 4.6\text{ mm}$). The components of the mobile phase, methanol and water in a ratio of 45:55, were filtered using a 0.2 μm membrane filter prior to use and were delivered from the solvent reservoir at a flow rate of 1 mL/min, resulting in a column backup pressure between 260 and 270 kgf/cm 2 . Each sample was measured in three separate replicates.

PHYSICAL APPEARANCE/VISUAL INSPECTION

The prepared extracts were evaluated based on their clarity, color, fragrance, and capacity to generate foam [2].

DETERMINATION OF pH

A pH meter was used to measure the pH of a 10 % v/v extract of soapnuts mixed with distilled water at room temperature [35].

DETERMINATION OF % OF SOLID CONTENTS

A cleaned, dried, and weighed evaporating dish was filled with 4 grams of extract. To confirm the accurate weight of the extract, the dish along with the shampoo was weighed again. The evaporating dish was placed on the hot plate to remove the liquid portion of the shampoo. Once the shampoo was fully dried, the weight and thus the percentage of the solid content that remained were calculated [4].

FOAMING ABILITY AND FOAM STABILITY

The cylinder shake method was utilized to evaluate foam content, where 50 mL of a 1 % soapnut extract solution was placed into a 250 mL graduated cylinder. The cylinder was then sealed with one hand and shaken ten times. After one minute of shaking, the total volume of foam produced was recorded. Foam stability was assessed by measuring the foam volume immediately after one minute and again after four minutes of the shaking test [18].

STATISTICAL ANALYSIS

The data analysis was using SPSS version 19. Each test was carried out three times, with the results presented as mean \pm standard deviation. To assess significance, a single-factor ANOVA was employed. A *p* value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

MORPHOLOGICAL CHARACTERISTICS OF FRUIT

The fruit size, color and shape are important characteristics, which help in differentiating the varieties of *Sapindus* fruit. Data recorded on 8 quantitative and 3 qualitative traits of three soapnut fruit (*Sapindus* spp.) species were presented in Table 1. The length and width were 1.823 ± 0.200 mm and 1.724 ± 0.180 mm for the

S. trifoliatus fruit (Fig. 1) respectively. Based on the means comparison, the highest mean value was observed in *S. trifoliatus* pulp weight with 1.173 ± 0.324 . Pulp thickness had size with 0.160 ± 0.048 in *S. trifoliatus*. The mean value of seed length, seed width and seed weight was observed in 1.473 ± 0.064 , 1.400 ± 0.118 and 1.719 ± 0.247 other research has also highlighted differences in the characteristics of plants and fruits among androgenic lines of the same origin. [15] Investigated the morphology and anatomy of fruits in 13 *Daucus* Species alongside four closely related non-*Daucus* groups. Their results revealed significant variation among the groups in terms of fruit size, shape, weight, surface texture, and specific anatomical features. The morphometric traits and weights of the fruits showed considerable differences not only among the groups but also within species. Consequently, the micromorphological traits of the fruit surface and the anatomical structure of the fruit appear to be more effective for distinguishing between species.

Table 1

Assessment of the morphological traits of *Sapindus trifoliatus*

S. No.	Morphological characteristics	<i>S. trifoliatus</i>
1	Fruit shape	globose
2	Fruit surface	smooth
3	Fruit color	brown
4	Ploidy level	n 18
5	Fruit length (cm)	2.369 ± 0.142
6	Fruit weight (g)	4.472 ± 0.734
7	Fruit width (cm)	2.044 ± 0.165
8	Pulp thickness (cm)	0.216 ± 0.036
9	Pulp weight (g)	2.793 ± 0.610
10	Seed length (cm)	1.473 ± 0.064
11	Seed width (cm)	1.400 ± 0.118
12	Seed weight (g)	1.719 ± 0.247

Each value of the column represents means \pm standard deviation and significantly different at $p \leq 0.05$ probability, significant differences at 0.05 and according to Duncan's multiple range tests.



Fig. 1. Morphological characteristics of fruits and seeds of *S. trifoliatus*.

FRUIT ANATOMY

The studied taxa's fruit wall (pericarp) demonstrated a standard three-layered structure: exocarp, mesocarp, and endocarp (Fig. 2), with thicknesses varying between 0.1 to 0.25 cm (Table 1). The exocarp, being a single layer, consisted of small, thick-walled cells that were mostly vertically rectangular; the section of the exocarp over the secondary ribs was made up of flattened rectangular cells. The mesocarp was formed from multiple layers of irregular thin-walled parenchymatic cells, which were usually larger than those found in the exocarp. The endocarp was formed by a single, compressed layer of somewhat lignified cells that typically adhered closely to the seed coat (Fig. 2). The pulp thickness was depending upon size of parenchymatic cells in mesocarp of fruit (Fig. 2). *S. trifoliatus* had larger parenchymatic cells. The pulp of *S. trifoliatus* is light brown in color.



Fig. 2. Pulbcross section of *S. trifoliatus*.

PHYSICAL APPEARANCE / VISUAL INSPECTION

The evaluation of soapnut extraction was based on physical attributes such as color, scent, and transparency (Table 2). Significant variations were observed in terms of scent, transparency, and foaming characteristics among the extracts of the three fruits. *S. trifoliatus* exhibited a clear, yellowish-brown hue and had a pleasant fragrance (Fig. 3, Table 2).

pH

Most shampoos are designed to have a neutral or slightly alkaline pH to minimize the risk of hair damage. Additionally, the pH of shampoo influences eye irritation levels, improves hair characteristics, and helps preserve the scalp's ecological balance [23]. The commercial shampoos analyzed were observed to have pH levels within the acceptable range of 5 to 7 [35]. The pH values of the *S. trifoliatus* extracts ranged between 7 and 5, as shown in Table 2.



Fig. 3. Physical appearance of *S. trifoliatus* extract.

% OF SOLID CONTENTS

Effective shampoos usually contain 20–30 % solid ingredients, which contributes to their ease of application and rinsing from the hair. A formula with insufficient solid content can become too liquid and wash away quickly, whereas too much solid content can make it challenging to spread through the hair or rinse out thoroughly. The solid content percentage in all tested extracts was determined to be between 22–25 %, indicating that they will rinse out easily (Table 2). Our results align with previous studies on the solid content percentage of herbal shampoos [4].

FOAMING ABILITY AND FOAMING STABILITY

Foaming or lathering is essential for consumers, making it an important criterion in the evaluation of shampoo. The foam volumes produced by *S. trifoliatus* (Fig. 4) were below 100 mL, specifically averaging 38.33 ± 12.6 mL (Table. 2). The foam produced by the *S. trifoliatus* extract was noted to be large, compact, uniform, airy, and less stable and displayed a foam volume after 5 minutes, indicating that its foam possesses less stability. The enhanced foaming characteristics of the formulated shampoo may be attributed to the blend of soapnut [31].

Table 2

Physicochemical evaluation of *S. trifoliatus* fruit extract

S. No.	Physicochemical characteristics	<i>S. trifoliatus</i>
1	Color	yellowish brown
2	Transparency	transparent
3	Odor	good

4	% Solid contents	20
5	pH	5.70±0.10
6	Foam volume (mL)	38.33±12.6
7	Foam type	small, airy
8	Saponin content (µg/mL)	0.676

Results are mean±SD ($n = 3$); *significant difference ($p < 0.05$) by Duncan's multiple range tests.



Fig. 4. Foaming ability and stability of *S. trifoliatum* extract.

QUANTIFICATION OF SAPONINS BY HPLC METHOD

The method using HPLC has successfully quantified the saponin levels. The HPLC-DAD technique proves to be an excellent method for assessing saponins in camote tubers, as it provides rapid analysis times, minimizes sample consumption, ensures high precision and accuracy, is economical, and is widely available in analytical laboratories [6, 10]. The peak areas of the standards and the corresponding peak areas of selected peaks in the sample were utilized to determine the saponin content in the samples. To create a standardized quantification method [33] compared the accuracy of reverse phase high performance liquid chromatography (HPLC) with vanillin-sulfuric acid and antimony pentachloride colorimetric assays. The major peak of saponin in the HPLC chromatograms were identified in *S. mukorossi* our HPLC analysis revealed that fruits of *S. trifoliatum* (Fig. 5) contained saponin B peak. HPLC conditions of three fruits can separate saponin B major peaks and major peaks in Standards peak A (Fig. 6) were eluted at 1.18 min and their corresponding compounds in the sample extract were detected at 1.20 min, respectively. The total content of saponins in fruits ranged from 0.676 µg/mL in *S. trifoliatum* (Table 2).

The calibration curve was fabricated using a saponin standard over a concentration range of 0.2–1.0 mg/mL. The regression equation of the calibration line was $y = 5.3 + 10^5x + 2000$ (where y represents peak area and x represents concentration). The correlation coefficient (r^2) was ≥ 0.99 , indicating good linearity. Each concentration level was analyzed in triplicate ($n = 3$), and the mean peak areas were used for regression analysis.

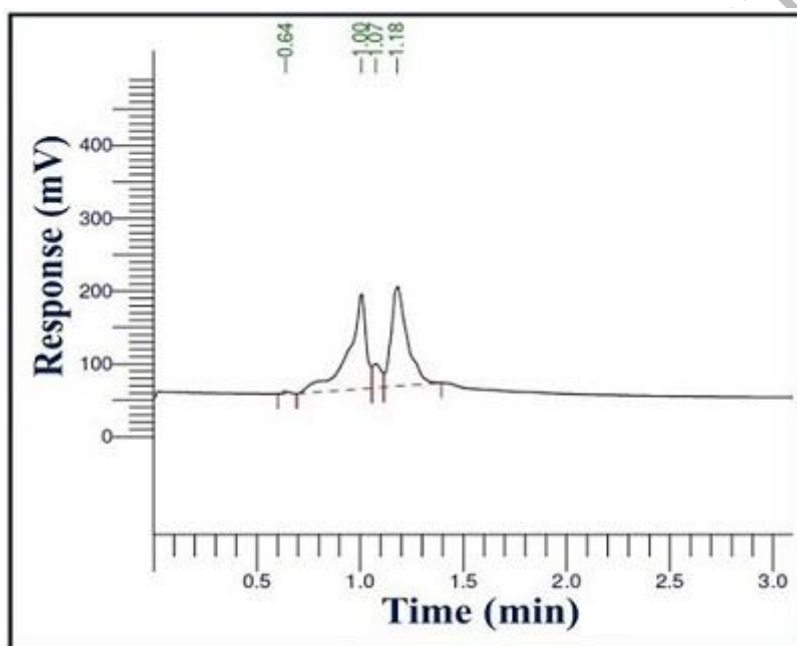


Fig. 5. HPLC chromatograph of standard (A 1.18).

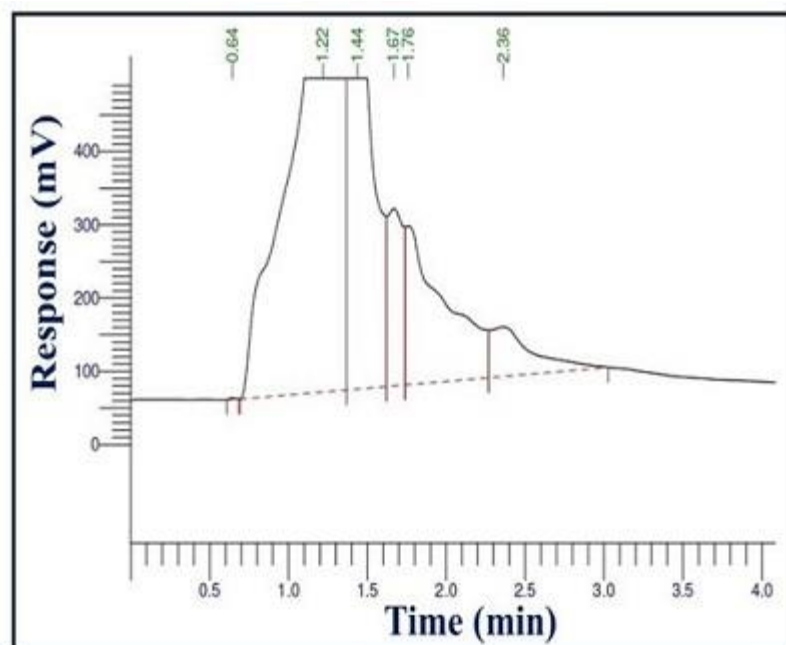


Fig. 6. HPLC chromatograph of *S. trifoliatus* (B 1.22).

CONCLUSION

This research provides in-depth insights into the morphology, anatomy, physicochemical properties, and total saponin levels of *S. trifoliatus* the findings presented detailed information about the morphological and anatomical traits of the fruit across the studied taxa and highlighted several key diagnostic features of the fruits, such as size, shape (ranging from ellipsoid to oblong), and weight, as well as the sculpture of the fruit's surface and certain anatomical traits, including the presence or absence and size of vittae, thickness of the pericarp, and configuration of the exocarp cells. This study broadens the knowledge of the fruits of *S. trifoliatus*. The HPLC analysis revealed total content of saponins in fruits ranged from 0.676 $\mu\text{g}/\text{mL}$ in *S. trifoliatus* the findings of the present study regarding morphology, anatomy and total saponin of three species fruits must be emphasized as fundamental data provided the information on the quality control for future cultivation of *Sapindus trifoliatus* in new places and breeding new varieties containing desired or higher amount of bioactive compounds.

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