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Characterization and screening of candidate plus trees of *Sapindus emarginatus* Vahl. for saponin content

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Abstract
Soapnut (*Sapindus emarginatus* Vahl.) is one of the most traded non timber forest product bioresource in Tamil Nadu. The dried fruit contains higher percentage of saponin, a substance rich in detergent properties. As a measure of sustainable consumption, soapnut harvesting in the wild should be minimized and compensated by agroforestry and industrial plants grown outside of the forests. To identify the superior source in terms of higher fruit yield and saponin percentage the germplasm was assembled and studies were undertaken at Forest College and Research Institute, Mettupalayam. A reconnaissance survey was carried out in Tamil Nadu to select *Sapindus emarginatus* Candidate Plus Trees (CPTs) based on the morphometric attributes of tree and fruiting output during peak fruiting season. Saponin content of selected Candidate Plus Trees were analysed and was found to range between 10.8-18.2%. Among the sources collected fruits collected from Kunjapanai-1 (TN SE 03) source recorded the highest saponin content (18.2%).

Keywords: Soapnut, saponin, pericarp, bioresource

Introduction
Sapindus emarginatus is an important indigenous plant with lots of traditional importance, belonging to the family Sapindaceae, capable of colonizing in dry shallow soils, loamy soils and lateritic soils (Swaminathan and Revathy, 2013) [15]. It is a medium-sized deciduous tree endemic to India's Southern states. It is considered to be originated in the Western Ghats and later extended up to the West Central North Indian biogeographical regions. Tropical and subtropical climate is favourable for the species. The flowers appear in large clusters during the month of October- December. Fruits ripen in the hot season from February to April. Among the oldest industrial and medicinal plants documented soapnut has good utility, due to their high functional qualities, bio- resource and environmental safety, and ecological adaptability. The soapnut is gaining popularity as natural and green surfactants. Saponins are secondary metabolites produced by numerous plants. (Goral and Wojciechowski, 2020) [4]. The fruit's pulp contains saponin, which when rubbed with water makes a thick lather. This is mostly utilized in the detergent and soap industries, which explains its common name of soapnut. Surfactants derived from renewable sources such as plants are an ecological alternative to synthetic surfactants (Wojton *et al.*, 2021) [17]

Soapnut, a non-timber forest product (NTFP), is crucial for forest dwellers and tribals for their livelihoods and sales. With the rise in demand for environmentally friendly forest products, there has been a surge in interest in NTFP collection and selling on a big scale for long-term development. The soapnut tree is found in both dry evergreen and deciduous belts with diverse climate and topography (Warrier *et al.*, 2011). Soapnut species with a wide geographic range are likely to show variation in their active principle i.e, saponins. Thus identification of elite trees through chemotyping for saponins would provide promising base material for raising quality planting stock and effective utilization of the potential bioresource. Saponins are high-molecular- weight glycosides that have a sugar moiety attached to a triterpene or steroid aglycone. (Anandlakshmi *et al.*, 2013) [2]. The distribution of saponin varies greatly amongst plant organs. The usage of saponins in herbal, cosmetic, and medicinal goods have a considerable market value. Pericarp triterpene saponins are widely used in traditional Indian and Thai medicine as antifertility, antipuritic, and anti-inflammatory medications (Srikanth and Muralidharan, 2010) [13]. In order to ensure the long-term sustainability of the priceless bioresource, this study investigates the standardization of saponin extraction and quantification of saponin in *Sapindus emarginatus* fruit pericarp and saponin variability in soapnut populations.

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The germplasm resources with extensive morphological variation can also contribute to *S. emarginatus* breeding in the future by facilitating development of new cultivars with high saponin yield. (Sun *et al*., 2017) [14]

Materials and Methods

Survey and Collection

An extensive reconnaissance survey was carried out to identify CPT's in Tamil Nadu, India. CPT's were selected and marked based on comparison tree selection method using the morphological features *viz*., height, girth at breast height, clear bole height, crown width, and number of branches. The selected trees are referred to as candidate plus trees (Zobel and Talbert 1984). The selected CPT's were given the accession numbers based on the region from which they were selected. The fruiting intensity was used as the selection criterion for Candidate plus Trees (CPT).

Extraction and estimation of saponin

The pericarp of the mature collected fruits was removed from the soap nuts. The pericarp was crushed after being air dried at room temperature. Saponin was measured using the Birk *et al*. (1963) method, as modified by Hudson and El-Difrawi (1979). One gram of the ground material was refluxed in 20 ml of 20% ethanol for 12 hours at 55 °C. The extract was filtered through Whatman No. 1 filter paper and the leftover material was repeatedly extracted with 20 ml of 20% aqueous ethanol until a pale-yellow colour solution was obtained. The combined extract was then concentrated to 20 ml and vigorously shaken twice with 20 ml diethyl ether in a separating funnel. The aqueous layer was saved and the ether layer was discarded. The pH of the aqueous solution was adjusted to 4.5 by adding NaOH/HCl and the solution was shaken with 20 ml n-butanol twice. The combined n-butanol extracts were washed twice with 5 ml of 5% aqueous NaCl and evaporated to dryness in a fume cupboard to give crude saponin which was weighed. The total saponins were calculated using a gravimetric technique and expressed as a percentage of total saponins.

$$\text{Saponin \%} = \frac{\text{Final weight of the residue (g)}}{\text{Weight of the sample taken (g)}} \times 100$$

Data Analysis

The data analysis were conducted in Completely Randomized Design. The data obtained is processed and saponin content were analysed by one way ANOVA at 5% level of significance using AGRESS software.

Results and Discussion

In the present investigation, wide range of variations were observed in growth characters of selected trees *viz*., height of tree (6 m to 10 m), girth at breast height (58 cm to 167 cm) and No. of branches (7 to 15) (Table 1). The geographical location and salient features of the CPT's are presented in the Table 1.

Among the selected CPT's saponin content obtained ranged between 10.8 to 18.20% (Table 2). The highest saponin percentage is found in TN SE 03 (18.20%) followed by TN SE 05 (18.00%) and TN SE 07 (16.80%). The least percentage of Saponin was found in TN SE 02 (10.80%). When saponin content was assessed location wise Kunjapanai-1 (TN SE 03) recorded the highest saponin (18.2%) followed by Kunjapanai-2 (TN SE 04) (18.00%) and Anthiyur (TN SE 07) (16.80%) and the least percentage of Saponin was found in Tiruvannamalai-2 (TN SE 02) (10.80%). The variations in the saponin could be attributed to both genetic influence and environmental interactions.

Table 1: Accession details of selected Candidate Plus tree (CPTs) of *Sapindus emarginatus*

Sl. No.	State	Place	Latitude	Longitude	Accession no.	Height (m)	GBH (cm)	Clear bole height (m)	Crown width (m)	No. of Branches
1	Tamil Nadu	Tiruvannamalai 1	12.6399	79.1659	TN SE 01	09	67	3.0	2	11
2	Tamil Nadu	Tiruvannamalai 2	12.5116	79.1226	TN SE 02	06	58	2.5	3	07
3	Tamil Nadu	Kunjapanai 1	11.3573	76.9320	TN SE 03	18	167	4.0	4	15
4	Tamil Nadu	Kunjapanai 2	11.3578	76.9298	TN SE 04	15	146	3.0	3	14
5	Tamil Nadu	Kunjapanai 3	11.3593	76.9317	TN SE 05	16	155	4.0	4	15
6	Tamil Nadu	Kunjapanai 4	11.3578	76.9298	TN SE 06	17	162	3.0	3	13
7	Tamil Nadu	Anthiyur	11.531954	77.5360	TN SE 07	11	167	5.0	4	12
8	Tamil Nadu	Dharmapuri 1	12.105047	78.1431	TN SE 08	09	64	3.0	2	10
9	Tamil Nadu	Dharmapuri 2	12.1055	78.1433	TN SE 09	06	59	2.5	3	07
10	Tamil Nadu	Hogenakal	12.1209	77.8181	TN SE 10	10	99	4.5	3	15
	Mean					11.7	114.4	3.45	3.1	11.9

Table 2: Saponin content of selected candidate plus trees (CPTs) of *Sapindus emarginatus*

Sl. No.	Source	Saponin content (%)
1	TN SE 01	13.80
2	TN SE 02	10.80
3	TN SE 03	18.20*
4	TN SE 04	16.60*
5	TN SE 05	18.00*
6	TN SE 06	14.00
7	TN SE 07	16.80*
8	TN SE 08	14.00
9	TN SE 09	16.60*
10	TN SE 10	15.60*
Mean		15.44
SEd		0.4945
CD (0.05)		1.0315

*Significant at 0.05%

Sun *et al*. (2017) [14] reported that the saponin content observed in *S. mukorossi* ranges from 7% to 27%, which is higher than *S. emarginatus* which ranges between 10.8 to 18.2% which is in corroboration with present study. Similar results were also indicated in research conducted by Anandlakshmi *et al*. (2014) [1], who registered that saponin content of S*apindus emarginatus* ranges from 10.44 to 20.33%. These results are in line with present studies. Bajad and Pardeshi, (2013) [3] isolated Saponin from *Sapindus emarginatus* fruit pericarp. In the study it was found that the saponin present in *Sapindus emarginatus* was 324.85 microgram/ml which was quantified using HPLC. Rijaji (2016) [11] extracted saponin from Kolowe (*Chydenanthus excelsus*) seeds. The content of saponin extract was greater than 32.6% in Kolowe seeds having great potential as a source of saponin. In similar studies, Variations in chemical composition of neem seeds from different agro climatic zones of a Gujarat has been documented by Gupta *et al*. 1998 [5]. Studies conducted in *Azadirachta indica* revealed that there was a significant variation for seed oil content and azadirachtin content of neem and it was found to be affected by climate and habitat (Kumaran, 1997) [8], Kala (2009) [12] studied the variation in bixin content (1.13% to 3.13%) of *Bixa orellana* seeds in the assembled genotypes. Hence it can be concluded that the functional indices of the fruit depends largely on the biotic & abiotic factors.

Conclusion

The superior source of higher saponin yielding has been identified in this study *S. emarginatus* tree improvement and breeding programme would be carried out. It would ensure higher financial gains for communities that depend on forest, tree farming as well as for soap and pharmaceutical industries. It also provides baseline for the sustainable usage and long-term preservation of the irreplaceable bioresource in the contex of climate change.

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