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Seed source variations for morpho-chemical traits of fruits in *Sapindus emarginatus* Vahl. from central western ghat locations of Karnataka, India

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Abstract
Sapindus emarginatus Vahl. or *Sapindus trifoliatus* L. belongs to family Sapindaceae. Fruits rind is rich in saponin. Fruit rind and seeds are harvested as non-timber forest products (NTFP) because of its traditional use and industrial raw materials for extraction of rich saponins and kernel fatty oil respectively. Plant based saponins have good demand mainly utilized for ecofriendly detergent, cosmetics, pharmaceutical applications like, antimicrobial, anti-inflammatory, antioxidant effects, immunomodulatory, anticancer, hepatoprotective, cholesterol-lowering and neuroprotective. Coefficient of variation (C.V.) between seed sources shown 18.82% for tree mean girth and 11.40% for tree height. Sagara location seed source recorded maximum fruit diameter and rind weight of 20.80 mm and 145.50 g. Similarly, highest percentage of saponin yield by KASE 05 (Sagara) seed source (19.80%) followed by KASE 01 (Athani) location sources with 19.03%. Minimum saponin yields was varied with KASE 08 (Madikeri) and KASE 10 (Gundlupete) location source. It was observed that R^2 value for rind weight v/s saponin yield was positive slope with correlation of 0.154 but, when saponin yield was correlate with altitude of seed sources it was shown negative slope. In present study rind weight was considered as principal component to correlated other fruit morpho-chemical traits of fruits in different seed sources. Fruit rind weight was strongly correlated with their respective fruit length, fruit dimeter, fruit weight and rind saponin yield %. Hence, fruit rind weight can be good estimate to morphological variation of fruit parameters.

Keywords: Non-timber forest products, seed source, morpho-chemical, rind saponin yield

Introduction
Species diversity in tropical forests holds paramount significance not only for the survival of species but also for its enhancement. It is imperative that a significant level of variability for economic traits exists within the population to facilitate profitable exploitation through breeding or selection. *Sapindus emarginatus* Vahl. belongs to family Sapindaceae. Fruit rind and seeds are harvested as non-timber forest products (NTFP) because of its traditional use and industrial raw materials for extraction of rich saponins and kernel fatty oil respectively. Plant based saponins have good demand mainly utilized for ecofriendly detergent, cosmetics, pharmaceutical applications like, antimicrobial, anti-inflammatory, antioxidant effects, immunomodulatory, anticancer, hepatoprotective, cholesterol-lowering and neuroprotective. Seed oil can be used for pharma and biodiesel purpose. Saponins are a class of chemical compounds found in various plant species. They are glycosides, consisting of a sugar moiety bonded to a triterpene or steroid aglycone. Saponins are known for their surfactant properties, which allow them to create lather and foam in aqueous solutions. This makes them useful in various cleaning and cosmetic applications. The importance of this species for global trade in soap and cosmetic industries due to the seed oil and saponin content extracted from leaves, fruits and seeds. (Swaminathan and Revathy, 2013) [1] (Wojton *et al.*, 2021) [2]. The world-wide trade of saponin is 650 million US$ in 2017 and in future expected to grow up to 970 million US$ in 2025. (Srikanth and Muralidharan, 2010) [3] Our current exporting status of saponin is approximately of 68,368 kg/year. Saponins mainly utilized for making detergent, cosmetic, pharmaceutical products, antimicrobial, anti-inflammatory, antioxidant effects, immunomodulatory, anticancer, hepatoprotective, cholesterol-lowering and

neuroprotective. *S. emarginatus* seed fatty oil is a versatile and valuable product with a wide range of applications, from cosmetics and medicine to industrial and agricultural uses. (Anandlakshmi *et al.*, 2013) [4]. Its natural properties offer benefits for skin and hair care, traditional medicine, and sustainable industry practices. As awareness of eco-friendly and sustainable products grows, the demand for natural oils like Sapindus seed oil is likely to increase, supporting environmental conservation and health-conscious consumer choices. Sapindus seed oil holds promise as a feedstock for biodiesel production due to its renewable nature and favourable fatty acid composition. While there are challenges to overcome, particularly in terms of yield and processing costs, continued research and development could make sapindus-based biodiesel a viable and sustainable alternative to conventional diesel. (Sun *et al.*, 2017) [5]. Utilizing sapindus seed oil for biodiesel not only supports renewable energy initiatives but also contributes to environmental conservation and economic development.

Materials and Methods

Collection of experimental material

Investigation of predominant *Sapindus emarginatus* population growing areas of Karnataka were identified consulting Karnataka Forest Department and matured fruits from 10 central western ghats districts of Karnataka population were collected during January- February 2023. Fruits were collected from 3 trees from each population and made pooled into one seed source. Hence, total 30 trees with 10 seed sources lots were collected for experiment. Selected seeds sources were given accession code as KASE 01 to KASE 10, and recorded latitude, longitude and altitude of the location were documented. Average girth, height of seed sources was recorded (Table 1). The fruits collected from each population were kept in separate open gunny bags and labelled with the date of collection, locality and information about the seed sources. For fruits morphometric traits measurements vernier calliper was used to measure fruit length, diameter. Further, electronic weighing balance was used to measure fruits weight and rind weight.

Estimation of saponin yield percentage in *S.emarginatus* fruit rind

For estimating saponin, the mature fruits were collected from selected seed sources tree. The rinds were crushed after being air dried at room temperature. Saponin was measured using the Birk *et al.* (1963) [6] method, as modified by Hudson and El-Difrawi (1979) [7]. Ten grams 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.

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

Results and Discussion

Mean growth attributes of Seed sources in *S. emarginatus* populations

Table 1 depicts with mean growth parameters of seed sources like tree mean girth and tree mean height showed significant difference. Coefficient of variation (C.V.) between seed sources shown 18.82% for tree mean girth and 11.40% for tree height. KASE 07 seed source recorded the highest mean girth of (1.80 m), followed by KASE 10 (1.65m) and lowest was in KASE 03 (1.05m). With respect to tree total height, highest was documented in KASE 10 (13.00 m) and lowest was in KASE 09 (8.50 m). Mainly variation in seed sources trees mean gith and height was due to altitude, rainfall and soil characters and genetic makeup of selected mother trees, like wise Hanumatha (2020) [8] identified girth (13.5 to 50.5 cm), tree height (2.0 to 8.5 m) and crown spread (1.0 m to 6.5 m) as most variable traits in *Cinnamomum zeylanicum*. He opined that these variations may be largely contributed by provenance locality factors *viz*. altitude, rainfall and soil characters and genetic makeup of selected mother trees. Manish (2020) [9] studied provenance effect on 36 plus trees of *Butea monosperma*. Traits *viz.*, total tree height (11.67 m - 8.23 m, C.V = 7.65%), diameter of tree (48.79 cm -26.33 cm, C.V = 3.36%), crown spread (7.95 m – 4.84 m) and bark thickness (11.67 mm – 7.33 mm) indicated highly significant differences among the locations, which revealed the existence of good deal of variability in the morphological traits. Abhijith (2018) [10] assessed 12 *Ailanthus triphysa* plus trees from three agroecological zones of Thrissur, Kerala. The results showed significant variation in height (19 m to 24 m), girth (120 cm to 143 cm) with coefficient of variation of 7.14% and 4.77% respectively.

Seed Sources variation for fruit morphometric and saponin yield % in *S. emarginatus*

Significant variation was recorded among seed sources for fruit length, fruit diameter, 100 fruit weight, 100 rind weight and fruit weight to rind weight ratio (Table 2). In group of ten seed sources maximum fruit length was documented in KASE 05 (23.05mm) followed by KASE 02 (21.45mm) was on par with maximum and minimum length for KASE 06 (15.35mm). With respect to fruit diameter maximum was observed in KASE 05 (20.80 mm) followed by KASE 02 (17.86 mm), KASE 01 (17.50 mm) were at par with maximum fruit diameter and lowest diameter for KASE 06 (13.40 mm). In case of 100 fruit weight highest was achieved by KASE 05 (305.00 g) followed by KASE 01 (280.45 g) and least was found in KASE 06 (228.25 g). For important component of fruit, significant highest 100 rind weight was recognized in KASE 05 (145.50 g) followed by KASE 03 (138.20 g) and lowest for KASE 04 (112.20 g). With an interesting derived factor fruit weight to rind weight ratio was maximum for source of KASE 02 (2.49) and minimum for KASE 09 (1.88). Ramachandran and Vasudeva (2020) [11] studied fruit traits variation in natural population of *Pyrencatha volubilis* in Karamanayar (Kerala) and Coromandel Coast (Tamil Nadu) and reported that Kizoor source is superior in all fruit trait than other 18 sources. Such wide variation in fruit traits across population have been reported by Zhang *et al*. (2021) [12] in *Juglans mandshirica*, Das *et al*. (2020) [13] in *Terminalia bellarica*, Pravin *et al*. (2020) [14] in *Schleichera oleosa*, Meenakshi *et al.* (2020) [15] in *Dalbergia sissoo,* Sudrajat (2016) [16] in *Anthocephalus cadamba.* Das *et al.* (2020) [13] conducted experiment on provenance variation in morphometric characters of fruit and seeds of *Terminalia bellarica*. Fruits collected from nine provenance of Odisha revealed that fruit length ranged from 2.21 to 3.87 cm, fruit width varied from 2.23 to 3.12 cm and 100 fruit weight ranged from 517.50 to 1340.17 g. The maximum fruit parameters like fruit weight (1340.17 g) and fruit length (3.87 g) were recorded from Kantama source, while maximum fruit width (3.12 cm) recorded from Deogan source. Zhang *et al.* (2021) [12] conducted experiment on morphological fruit variability among six provenances of *Juglans mandshirica* in Eastern Liaoning of China. Study reported that great variation found in most of the traits, especially shell thickness (25.10%) and nut sutural thickness (28.82%) showed huge variations. The average nut longitudinal diameter (41.21 mm), nut lateral diameter (27.85 mm), nut transverse diameter (27.37 mm), mean diameter (31.68 mm), nut weight (10.03 g), kernel weight (1.69 g), shell thickness (0.9 mm), nut sutural thickness (5.29 mm), kernel percentage (17.02%), and index of roundness (0.70). Highest kernel weight (1.91 g) kernel percent (19.1%) observed from Dadong source, highest nut weight (1.18 g) was accounted from Fushun provenance which produces a precious variety of fruit traits. It is evident that fruit variation is usual irrespective of species among different sources.

Table 2 data result showcasing sources, rind saponin percentage significant variation was observed. For fruit rind saponin highest achieved by source of KASE 05 (19.81%), KASE 01 (19.03%) on par with highest and lowest was recorded for KASE 08 (14.30%), KASE 10 (14.30%). Saponin content is commonly known as nonvolatile, surface-active compounds that are widely distributed in plants. Figure 1, showcasing the linear relationship between seed sources altitude and saponin yield percentage, i.e., weak positive correlation with negative slope was observed. In Figure 2, representing of linear relationship between rind weight and rind saponin yield %, depicts relatively positive correlation with positive slope indicated genotypic influence and environmental variation for differences in morpho-chemical characters between seed sources. Saponin have a diverse range of properties, including foaming and emulsifying (Price *et al.*, 1987) [17], pharmacological and medicinal properties (Attele *et al.*, 2002) [18], hemolytic properties (Oda *et al.*, 2000 [19]; Sparg *et al.*, 2004 [20], as well as antimicrobial, insecticidal, spermicidal and molluscicidal activities (Huang *et al.*, 2008; Sparg *et al.*, 2004; Saxena *et al.*, 2004) [21, 20, 22]. Saponins have found wide applications in beverages and confectionery, as well as in cosmetics (Price *et al.*, 1987) [17]. Plants in different geographic regions often have varied adaptations due to differences in latitude. A wide range of external stimuli can cause changes in plant cells, triggering a chain reaction that results in the creation and accumulation of alkaloids, which is also the cause of different adaptations (Liu *et al.*, 2022) [23]. The intra-specific variation in alkaloids production in plants has been reviewed and explained by genetic drift, relaxed selective pressure, introgression of traits through hybridization, gene pleiotropic effects and phenotypic plasticity. There is a quantitative variation in alkaloids not only between wild and cultivated plants, but even across plants from the same wild population (Demasi *et al.*, 2018) [24]. Similarly, many authors have reported secondary metabolite variation within species as well as between populations; Glucosinolates in Arabidopsis thaliana (Arany *et al.*, 2008) [25]. Phenolics in Achillea millefolium (Benetis *et al.*, 2008) [26]. Metabolite variation in different tissues of *Nicotiana attenuata* has been reported (Kim *et al.*, 2011) [27] and authors also stated that different plant tissues are defended in accordance with their value to the plant, costs of defense and risk of herbivore or pathogen attack. The variations in the saponin could be attributed to both genetic influence and environmental interactions. Sun *et al.* (2017) [5] 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) [4], who registered that saponin content of *Sapindus emarginatus* ranges from 10.44 to 20.33%. These results are in line with present studies of Bajad and Pardeshi, (2013) [28] isolated saponin from *Sapindus emarginatus* fruit pericarp and found that the saponin present in *Sapindus emarginatus* was 324.85 microgram/ml which was quantified using HPLC. Rijaji (2016) [29] extracted saponin from *Chydenanthus excelsus* seeds. The content of saponin extract was greater than 32.6% in *Chydenanthus excelsus* 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 [30]. 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 and Kala, 2012) [31], Kala *et al*. (2009) [32] 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 depend largely on the biotic and abiotic factors.

Correlation matrix (Pearson) for fruit traits of *S. emarginatus*

Table 3 depicts correlation coefficient shown positive and significant correlation for all fruit traits selected for present experiment. Correlation coefficients fruit length, fruit diameter, fruit weight and rind weight were strong positively correlated each other. Rind weight to rind saponin yield percentage was shown strong correlation. The traits considered were allometrically associated with each other, hence there was significant association. Total fruit weight was very strong correlated to other fruit traits, but fruit contained seeds as external governing factor for fruit weight. saponin yield % was extracted only from rind. In present study rind weight was considered as principal component to correlated other fruit morpho-chemical traits of fruits in different seed sources. Fruit rind weight was strongly correlated with their respective fruit length, fruit dimeter, fruit weight and rind saponin yield %. Hence, fruit rind weight can be good estimate to morphological variation of fruit parameters.

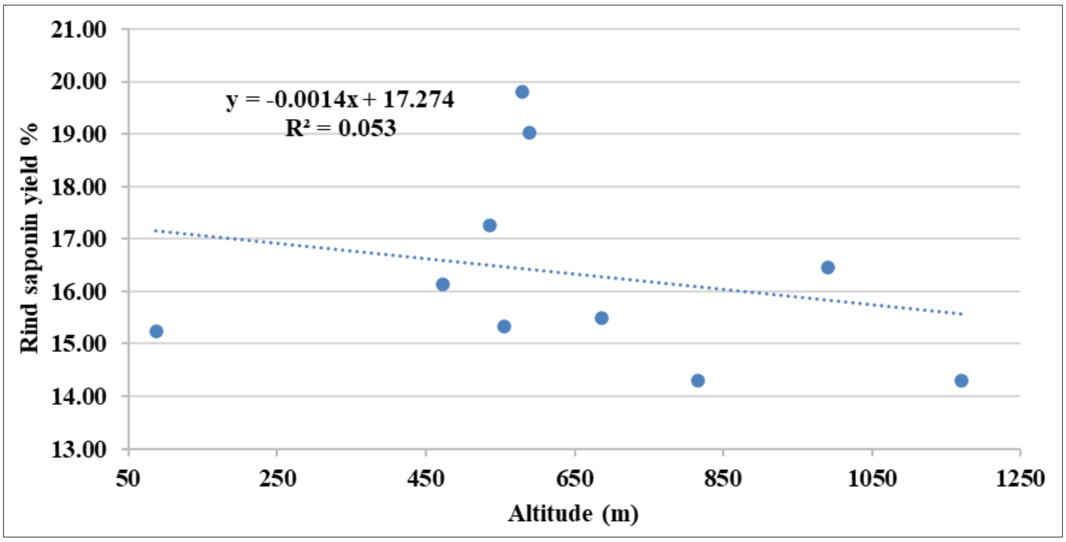

Fig 1: Linear relationship between seed sources altitude and saponin yield %

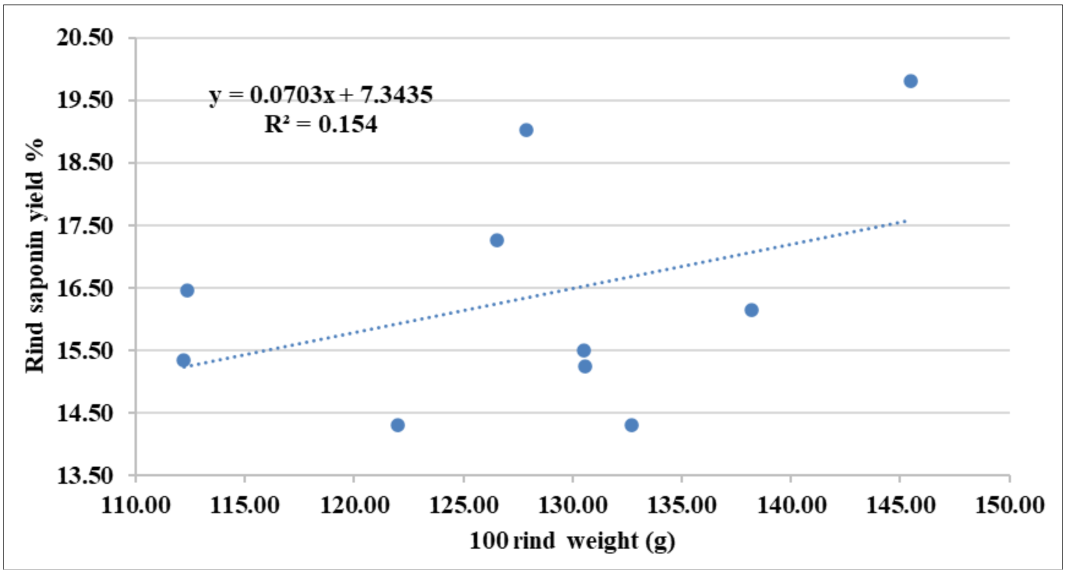

Fig 2: Linear relationship between 100 rind weight and rind saponin yield %

Table 1: Location, Accession code and mean growth attributes of Seed sources in *S. emarginatus* populations

Location	Districts	Accession code	Latitude	Longitude	Altitude	Mean GBH (m)	Mean Height (m)
Athani	Belagavi	KASE 01	16°35'21.7"N	74°56'19.8"E	590.00	1.35	12.00
Kalghatgi	Dharwad	KASE 02	15°10'37.3"N	74°47'13.7"E	536.00	1.15	10.50
Dandeli	Uttara Kannada	KASE 03	15°12'12.3"N	74°36'43.2"E	472.00	1.05	9.50
Hanagal	Haveri	KASE 04	14°48'57.0"N	75°01'16.3"E	555.00	1.10	10.00
Sagara	Shivamogga	KASE 05	14°07'05.3"N	74°58'38.9"E	579.00	1.60	11.50
Mudigere	Chikkamagaluru	KASE 06	13°11'22.9"N	75°33'55.7"E	990.00	1.10	10.20
Puttur	Dakshina Kannada	KASE 07	12°45'32.0"N	75°10'23.6"E	87.00	1.80	10.50
Madikeri	Kodagu	KASE 08	12°25'09.9"N	75°38'56.5"E	1170.00	1.20	11.00
Saraguru	Mysore	KASE 09	11°59'58.9"N	76°21'47.3"E	686.00	1.44	8.50
Gundlupete	Chamarajanagar	KASE 10	11°48'01.7"N	76°31'01.1"E	816.00	1.65	13.00
					Mean	1.33	10.67
					SEm(±)	3.10	0.78
					CD@5%	9.43	2.35
					CV (%)	18.82	11.40

Table 2: Seed Sources variation for fruit morphometric and saponin yield % in *S. emarginatus*

Accession code	Fruit length (mm)	Fruit diameter (mm)	100 fruit weight (g)	100 Rind weight (g)	Fruit: Rind ratio	Rind saponin yield %
KASE 01	19.27	17.50	280.45	127.86	2.19	19.03
KASE 02	21.45	17.86	275.00	126.55	2.49	17.25
KASE 03	18.33	15.80	267.68	138.20	1.94	16.14
KASE 04	15.40	13.89	230.12	112.20	2.05	15.33
KASE 05	23.05	20.80	305.00	145.50	2.10	19.81
KASE 06	15.35	13.40	228.25	112.35	2.03	16.46
KASE 07	17.35	15.32	250.14	130.55	1.92	15.24
KASE 08	17.77	15.30	235.16	122.00	1.93	14.30
KASE 09	18.25	16.55	245.00	130.54	1.88	15.50
KASE 10	19.45	17.25	260.00	132.70	1.96	14.30
Mean	18.57	16.37	257.68	127.85	2.05	16.34
SEm(±)	1.18	1.15	5.77	2.86	0.05	0.41
CD@5%	3.52	3.43	17.15	8.59	0.17	1.24
CV (%)	12.31	12.44	9.11	7.73	4.57	3.24

Table 3: Correlation matrix (Pearson) for fruit traits of *S. emarginatus*

Fruit morpho-chemical traits	FL	FD	FW	RW	RS
Fruit length (FL)	1.00				
Fruit diameter (FD)	0.97	1.00			
100 fruit weight (FW)	0.91	0.93	1.00		
100 rind weight (RW)	0.78	0.81	0.82	1.00	
Rind saponin (RS)	0.60	0.65	0.78	0.62	1.00

Conclusion
In concise Sagara and Athani seed sources shown greater fruit and weight sizes and also for yielded higher amount of saponin % was in Sagara and Belagavi sources compare to all other seed sources. Identification of superior seed source of higher saponin yielding has been made by above study in Karnataka. Succeeding to this study *S. emarginatus* kernel oil was quantified with chemical profiling by using Gas Chromatography/Mass Spectrometry. A well-structured assessment of early vigour progeny performance was carried out for tree improvement and breeding program at College of Forestry, Sirsi. It would ensure higher livelihood improvement for communities that depend on forest, tree farming as well as for financial return for soap and pharmaceutical industries. It also provides for further screening and establishment of germplasm multi location trails could help baseline for the sustainable usage and long-term preservation of the valuable bioresource in the context of deforestation.

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