National Conference on Plant Metabolomics (Phytodrugs – 2014)
Journal of Chemical and Pharmaceutical Sciences ISSN: 0974-2115

CHEMOTYPING OF *SAPINDUS EMARGINATUS* POPULATIONS FOR SAPONINS

Anandalakshmi R*, Srinivasan R, Vamadevan T, Suresh Kumar K, Krishna Kumar N
Division of Seed Technology, Institute of Forest Genetics and Tree Breeding, Coimbatore – 641002, Tamil Nadu.
***Corresponding Author:** E.Mail: lakshmir@icfre.org

ABSTRACT

Sapindus emarginatus Vahl., commonly known as Soapnut, is a valuable bioresource that finds importance as a Non-Timber Forest Produce (NTFP) in Tamil Nadu. The dried fruit rind is the most valuable part as it contains saponin, which is rich in detergent properties. As a measure of sustainable utilization, harvesting of soapnut in the wild need to be minimized and compensated by agroforestry plantations and industrial plantations raised outside the forests. With this objective, domestication and improvement of the *S. emarginatus* was undertaken at the Institute of Forest Genetics and Tree Breeding, Coimbatore. The tree improvement program of this species involved reconnaissance survey in Tamil Nadu to select Candidate Plus Trees (CPTs) of *S. emarginatus* based on the fruit yield during the peak fruiting season. Each CPT was chemotyped for saponins. Fruits collected were processed and the rind was used for saponin extraction. The method of isolation of saponins was standardized by differential solvent extraction method. The isolated saponins were quantified tree-wise by gravimetric method. The CPTs were shortlisted and ranked based on saponin yield (>13%). The high saponin yielding trees were multiplied on large scale for establishing trials and popularization among farmers and foresters.

KEYWORDS: Sapindus, soapnut, saponins, differential extraction, gravimetry

1. INTRODUCTION

Sapindus emarginatus or soap nut tree belongs to the family Sapindaceae. It is a medium-sized deciduous tree native to South India. It is found both in wild and introduced in tropical and sub-tropical regions. Soapnut tree is largely cultivated for its fruit. The pulp of the fruit contains saponin which produces rich lather when rubbed with water. This is largely used by detergent and soap industries and this fact reflected its common name as soapnut. Soapnut being a Non-timber forest product (NTFP) plays an important role in livelihood of forest dwellers and tribals and fetches Rs.90 per kilogram in the local market. With increase in use and demand for environmentally based forest products has led to increased interest in NTFP collection and marketing it in the large scale for sustainable development.

The soapnut tree is distributed both in dry evergreen and deciduous belts encompassing varying climate and topography (Warrier *et al.,* 2011) and hence is usually known to have local populations adapted to the environment. Soapnut species with broad geographic range has high probability to show variation in its active principle, saponins, and hence 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 consisting of sugar moiety linked to a triterpene or steroid aglycone. The saponin distribution among the plant organs varies considerably. The saponin content in soapnut varies from 10 to 18%. Saponins are of high market value and finds use in herbal, soap, pharmaceutical and photo film industry (Naidu *et al*., 2000). The photo film industry alone requires about 6 tonnes of saponin per annum; presently being totally imported (Mukesh Ahuja., 2006). It has been reported that in traditional Indian and Thai medicines the pericarp triterpene saponins are commonly used as antifertility, antipruritic and anti-inflammatory agents. Presently the demand for saponin is very high and the market price of 1Kg of soapnut is about Rs.875 in the international market. This paper discusses standardization of extraction and estimation saponins in fruit rind of *Sapindus emarginatus,* variability in soapnut populations for saponins, and the utilization of the results for the sustainable utilization of the valuable bioresource.

2. METHODOLOGY

Reconnaissance survey was conducted in Tamil Nadu to identify populations of *Sapindus emarginatus* especially in dry and moist deciduous forests at Mettupalayam, Palani, Thengumarada, Pillur, Thirumoorthi Hills, Sarkarpathy, Coimbatore, Hogenakal and Thalavadi. High fruit yielding Candidate Plus Trees (CPTs) were identified in each population. The fruits were pooled population wise and used for extraction, estimation and profiling of saponins. Each population is considered as a treatment for data analysis. The details of the populations are as follows, 1- Pillur, 2-Thengumarada, 3-Mettupalayam, 4- Palani, 5-Thalavadi, 6-Thirumoorthi Hills, 7-Sarkarpathy, 8-Aliyar and 9-Hogenakal.

2.1. Selection criteria: The selection criterion for Candidate plus Trees (CPT) was arrived based on the fruiting intensity. The individuals having 50 fruits and above per metre of the fruiting branch were selected as CPTs. The passport data of the CPT comprising the location, latitude, longitude, height, girth at breast height, number of fruits per foot length, fruit yield were recorded. The CPTs were selected by comparison tree method.

2.2. Saponin extraction and estimation: The mature fruits were collected and the rind was separated from the soap nuts (Fig 2). The rind was air dried at room temperature and powdered .Saponin was determined using the method of Birk *et al.* (1963) as modified by Hudson and El-Difrawi (1979). 1g of the ground sample was refluxed with 20ml of 20 % ethanol for 12 h at 55°C. The solution was then filtered using Whatman No.1 filter paper and the residue was re-extracted with 20 ml 20% aqueous ethanol repeatedly until it gave a pale colour solution. Then combined extract was concentrated to 20 ml and washed twice with 20 ml diethyl ether in a separating funnel by shaking vigorously. The aqueous layer was recovered and 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 a crude saponin which was weighed. The total saponins was determined by gravimetric method and expressed in percentage.

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

2.3. Ranking of CPTs based on saponin content: Based on the saponin yield from each CPT, the trees were ranked. The threshold limit for ranking the trees was saponin content of 13% and above. Seeds from these shortlisted high saponin yielding trees were used for germination and the seedlings were tested for germination percentage and seedling vigour.

2.4. Germination test: Germination test was conducted on sand medium in the nursery (32 ± 2°C; RH: 65 ±2 %) (ISTA, 1999). The test was carried out with 4 replications of 50 seeds each. Seeds were sown after removal of the rind or pericarp. The germination count was taken till 30 days since the day of first emergence. The germinated seedlings were transplanted to polybags filled with sand, red earth and farm yard manure in the ratio 1:2:1 after 45 days of germination. The seedlings in polybags were hardened for minimum period of 12 months before field planting.

2.5. Seedling Vigour Index: The vigour index was computed as per the method suggested by Abdul Baki and Anderson (1973) based on seedling height and germination percentage.

2.6. Statistical analysis: The tests were conducted in Completely Randomized Design. The effect of population on germination, seedling vigour and saponin content were analysed by one way ANOVA at 5% level of significance using GENSTAT 5.0 software.

2.7. Establishment of field trials: The seedlings were hardened and outplanted as field trials in three locations namely, Gudalur near Chennai, Kurumbapatti near Salem and Neyveli. The growth perfrmance of the trials are being monitored. The best performing CPTs are recommended as source for raising plantations.

3. RESULTS AND DISCUSSION:

Saponin a widely distributed compound in the pericarp of the soapnut was estimated gravimetrically for all the individual trees selected from nine different populations. Statistically significant variability was found to prevail in saponin content across populations at 5% level of confidence (Table 1). The saponin content in the pericarp of sopanut was found to range from 10.44 to 20.33 % within the nine populations studied. The population mean for saponins was determined to be 13.32%. Location- wise Sarkarpathy holds the highest saponin percentage of (20.33%) followed by Thalavadi (16.60%), Thengumarada (14.95%) and Palani (13.09%) which are above total average saponin percentage of the selected CPTs. Whereas, as, the populations such as Mettupalayam (12.69%), Pillur (12.67%) Hogenakal (12.20%), Aliyar (11.70%) and Thirumoorthi hills(10.44%) scored total average saponin percentage. The reasons for variations in the saponins could be attributed due to both genetic influence and environmental interactions. In similar studies, variations in chemical composition of neem seeds from different agro-climatic zones of Gujarat has been documented by Gupta *et al.* (1998).

Kaushik and Vir (2000) in a study for the selection of neem trees based on their biochemical composition, observed significant variability in individual fatty acids in 60 seed samples of neem collected from different provenances of Rajasthan. Reports on biochemical variation in other species include variation in free proline content in provenances of *Acacia nilotica* (Bagchi and Singh, 1994), terpenes in *Picea pungens* (Hanover, 1974), proline content and Superoxide dismutase activity among provenances of *Casuarina equisetifolia* (Reddy *et al.,* 2001).

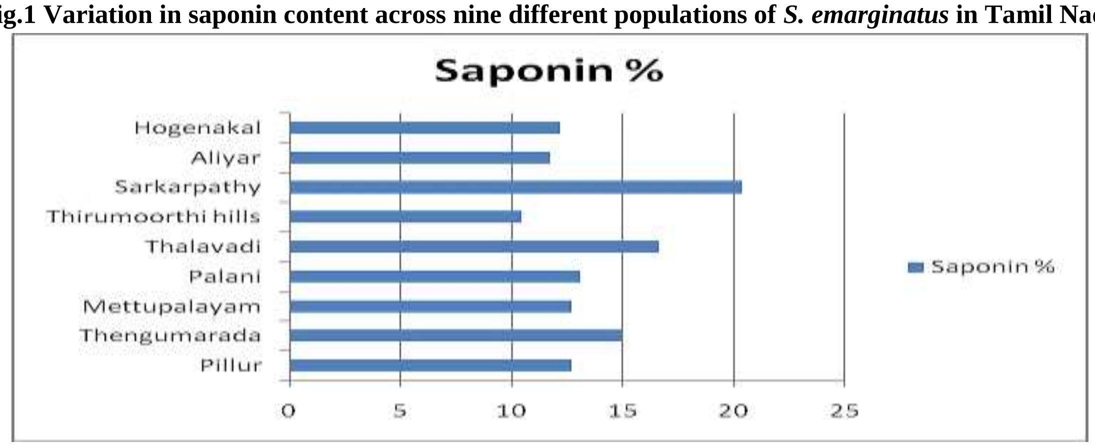
Studies on oil content in *Jatropha curcas* by Anandalakshmi *et al.* (2009) showed variation from location to location in the range of 32.39% to 24.80% oil. Owing to large variations in *Jatropha curcas* for oil content, FAO (2010) recommended testing over time and in different locations to determine the relative influence of genetic and environmental factors. In the present study, based on the results obtained, the CPTs scoring 13% and above for saponin content were shortlisted for mass multiplication and multilocation trials. Therefore this study reveals that saponin rich sources namely, Sarkarpathy, Thalavadi, Thengumarada and Palani are ideal sources for mass multiplication (Fig 1). From multilocation field trials further information on site-specific CPTs and stable CPTs could be generated and this would strengthen the improvement of soapnut and its popularization.

Table 1. Effect of populations on germination percentage, seedling vigour index and saponin percentage in *S. emarginatus*

Population	Germination %	Seedling Vigour Index	Saponin %
Pillur	53.8	1217	12.67
Thengumarada	59.8	1309	14.95
Mettupalayam	61.7	962	12.69
Palani	54.4	1313	13.09
Thalavadi	31.2	756	16.60
Thirumoorthi hills	47.3	453	10.44
Sarkarpathy	61.0	783	20.33
Aliyar	50.3	399	11.70
Hogenakal	58.0	364	12.20
S.e.d.	12.18	297.9	1.923
L.S.D.	24.23	592.7	3.825
Grand mean	55.4	1000	13.32

The study revealed no significant variations for germination percentage across the nine populations, while the seedling vigour index was strongly influenced by *S. emarginatus* population. The mean germination percentage and seedling vigour index across populations was found to be 55.4% and 1000 respectively. From the germination result it could be inferred that seed viability staus in *S. emarginatus* does not vary for populations that come from different agroclimatic zones and hence location specific pretreatments to enhance germination capacity is not necessary as in the case of teak seeds. However it is found that the potential of the soapnut seed to produce superior quality planting stock which is indicated by seedling vigour index, is governed by the source or population. In depth studies including Path analysis studies could deduce the genetic and environmental influence of population on these parameters.

Fig.1 Variation in saponin content across nine different populations of *S. emarginatus* in Tamil Nadu



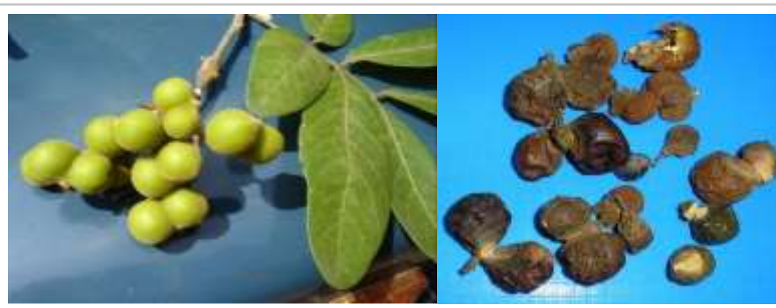


Fig.2 Fruits and dried rinds of *S. emarginatus*

4. CONCLUSION

Though tree improvement and breeding of *S. emarginatus* for saponins is a long term and cumbersome program, the study is very timely and important in the aspect of promoting an indigenous species of NTFP value and ecological significance. The results of the study besides ensuring higher econmic gains to the tree growers and forest dwelling communities, it would vouch for the suatainable utilization and long term conservation of the valuable bioresouce in the era of climate change.

REFERENCES

Abdul Baki, A. A. and Anderson, J. D. 1973. Vigour determination in soybean seeds by multiple criteria. Crop Science, 13: 630-633.

Anandalakshmi, R., Warrier, R.R., Sivakumar, V. and Singh, B.G. 2009. Fruit pericarp colour: influence on oil properties in *Jatropha curcas* (L.). South Asian Journal of Socio-Political Studies, 10(1):109-111.

Bagchi, S.K. and J. Singh. 1994. Provenance variation in *Acacia nilotica*- free proline content in leaves. Indian Forester, 6: 529-532.

Birk Y, Bondi A, Gestetner B, Ishaya IA (1963).Thermostable hemolytic factor in soybeans. Natural 197: 1089-1090.

FAO, 2010. Jatropha: A Smallholder Bioenergy Crop-The Potential for Pro-Poor Development. Integrated Crop Management 8.

Gupta. P.K., Tripathi, Y.C. and Rathore, M. 1998. Variations in chemical composition of seeds of neem (*Azadirachta indica* A. Juss.) from different agroclimatic zones of Gujarat. Journal of Non-Timber Forest Products, **5**(12): 10-13.

Hanover, James W. 1974. Biochemical analysis of tree speciation. In: C. P. P. Reid and G. H. Fechner (Eds.) Proceedings of the Third North American Forest Biology Workshop. September 9-12, 1974, pp. 106-131. Colorado State University, College of Forestry and Natural Resources, Fort Collins, 388 p.

Hudson, B.J.F. and El-Difrawi, E.A. (1979). The sapogenins of the Seeds of Four Lupin Species. J. Plant Foods 3:181-186.

ISTA. 1999. International rules for seed testing. Seed Science and Technology 27: 30-35.

Kaushik, N. and Vir, S. 2000. Variations in fatty acid composition of neem seeds collected from the Rajasthan State of India. Biochemical Society Transactions, **28**(6): 880–882.

Mukesh Ahuja, 2006. Life Science volume -2, pg. 269

Naidu, C. V., Rajendrudu G. and Swamy, P.M. 2000. Effect of plant growth regulators on seed germination of *Sapindus trifoliatus* Vahl. Seed Science and Technology, 28: 249-252.

Reddy, R. A., Chaitanya, K.V. and Desingh, R. 2001. Variation in photosynthesis and drought adaptive characteristics among *Casuarina equisetifolia* provenances. **In**: K. Gurumurthi, A.Nicodemus and Siddappa (*Eds*.) Casuarina in India- improvement and utilization. Institute of Forest Genetics and Tree Breeding, Coimbatore, India, pp. 81-87.

Warrier, R.R. 2012. *Sapindus emarginatus* Vahl. In : N. Krishankumar, K. Palanisamy, Maheshwar Hedge, Kannan C.S. Warrier and M. Krishnamurthy (eds.) Manual of economically important forestry species in South India. IFGTB Publications, Coimbatore, pp.395-400.