

## A Critical Review on Medicinally Important Oil Yielding Plant Laxmitaru (*Simarouba glauca* DC.)

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### Abstract

*Simarouba glauca* has a long history in herbal medicine in many countries. *Simarouba glauca* is one of the important herbal drug used against dysentery hence its bark is also known as dysentery bark. The bark and leaf extract of *Simarouba* is well known for its different types of pharmacological properties such as haemostatic, antihelmenthic, antiparasitic, antidysentric, antipyretic and anticancerous. The bark is used to cure fever, malaria, stomach and bowel disorders, haemorrhages, ameobiasis as well as leaf, fruit pulp and seeds are possessing medicinal properties such as analgesic, antimicrobial, antiviral, astringent emmenagogue, stomachic tonic and vermifuse. The crushed seeds are used as antigo against snake bites. The crude drug contents and active principles such as glaucarubin, quassinoids, ailanthinone, benzoquinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sistosterol. These are mainly involved in pharmacological activities of this plant. The present review summarizes pharmacological, Ethnobotanical Phytochemical aspects as well as nursery practices of this medicinal plant.

**Key words;** antihelmenthic, glaucarubin, pharmacological, quassinoids, simaroubidin vermifuse.

### I. Botanical description-

#### A. About *Simarouba glauca* DC.

A rainfed wasteland evergreen edible oil tree, *Simarouba glauca*, is commonly known as 'Laxmitaru' or 'paradise tree' belonging to family *Simaroubaceae*. The specific name *glauca* means covered with bloom which refers to the bluish green foliage. It is derived from Greek word 'glaukos' (bluish) [ICRAF Agroforestry Tree Database, 2007 <http://ecocrop.fao.org/ecocrop/srv/en/cropView?id=9785>].

Synonyms for *S. glauca* are:-*Simarouba medicinalis* Endl. And *Simarouba officinalis* Macfad.

Common names for *s. glauca*

English: - bitter ash, bitter damson princess tree, *Simarouba*, Paradise tree.

Spanish: - acajou blanc, daguilla, daguillo gabilan, juan, primero, laguilla, olivio, palo amargo.

Creole: - bwa blan, bwa fwenn, doliv fwenn.

French: - bois amer, bois blanche, bois frene, bois negresse, quinquina d Europe.

Trade name: - *Simarouba*, Dysentery bark, Mountain Damson, Acituno

[ICRAF Agroforestry Tree Database, <http://ecocrop.fao.org/ecocrop/srv/en/cropView?id=9785> 2007, 1]

### B. Distribution

#### a. Global Distribution

*S. glauca* DC. is indigenous to Southern Florida, the West Indies and Brazil [2]. It is native to Bahamas, Costa Rica, Cuba, EI-Salvador, Guatemala, Haiti, Honduras, Jamaica, Mexico, Puertorico, united states of America. While exotic to India, Srilanka, Phillippines and Myanmar [ICRAF Agroforestry Tree Database, <http://ecocrop.fao.org/ecocrop/srv/en/cropView?id=9785> 2007]. It grows under tropical conditions in Central America spreading from Mexico to Panama Southern Florida as well as Caribbean Islands. *S. glauca* was introduced in Kenya and Burundi in Africa in 1957 [3].

#### b. Distribution in India

It was first introduced by National Bureau of Plant Genetic Resources in the research station at Amravati in Maharashtra in 1966 [4, 5, 6] and to the university of Agricultural Sciences, Bangalore in 1986. In India it is cultivated in Orissa, Maharashtra and also at introductory stage of plantation in other states like Anand (Gujarat) Jodhpur (Rajasthan) Andhra Pradesh, Karnataka, Tamilnadu, West Bengal and Bhubaneswar (Orissa) [3]. Systematic Research and developmental activities on *S. glauca* began from 1992 onwards Lele

[<http://www.svlele.com/Simarouba.htm>. 2010] Now the cultivation of *S. glauca* spread to semiarid dry and saline land areas of other Indian states like Gujarat, Tamilnadu, Maharashtra, Karnataka and Andhra Pradesh. *S. glauca* tree has an ability to grow well even in marginal wastelands or dry land with degraded soil [7].

### c. General features of *S. glauca*

This is evergreen tree grows to a height of 12-15 m with large circular crown [8]. *S. glauca* occurs as an understory shade tolerant tree, commonly growing under the canopy of large fruit trees where birds perch and deposit the seeds. It is found to occur as associate with subtropical moist forest plants [ICRAF Agroforestry Tree Database,

<http://ecocrop.fao.org/ecocrop/srv/en/cropView?id=9785> 2007;]. The root system is shallow suitable for mountain soils. Stem is up to 9m height with 40-50 cm in diameter. It has finely cracked and grey colored outer bark while inner bark is creamy in color [9]. Leaves are pinnately compound with 3-21 leaflets oblong and often notched or smooth at apex; alternate, even, bluish oily green. Inflorescence is terminal panicle with ultimate branches producing dichasial cyme. Flowers are bisexual inconspicuous, calyx is green in color and dome shaped united with variable number of sepals. Petals are present in single whorl creamy greenish or yellowish creamy in color. The staminate flowers are with gynophores but without carpel with single ovule. The *S. glauca* exhibits considerable range of variations in floral characteristics and is considered as polygamo-dioecious [1, 10]. Armour [1] and Panhwar [<http://farzanapanhwar.blogspot.com/2007/08/simarouba-galauca-new-forest-plant-in.html> 2007], noticed floral variations such as pistillate form (female tree) staminate form (male tree) and andromonoecious form; tree having hermaphrodite flowers on several branches of the same tree. It is noticed that only 50% of trees are productive and that the remaining flowers are staminate or male.

It starts to induce flowers after attaining age of 6-8 years. Flowering is annual formed during spring, and produces in December and continuing up to the end of February. Several fruits are oval to oblong ovate drupes found to occur in groups of two to five. The fruits are purple in color having sweet edible pulp but slightly astringent in taste after ripening. The seeds are 1.5 to 2 cm. long pinkish or yellowish in color after ripening [11]. There are two varieties on the basis of fruit color one produces greenish white fruit and other violet to almost black fruits [12]. Jackals, Procupines, squirrels, cuckoos, mynas, bulbuls and other animals eat sweet pulp of drupelets and help in natural regeneration and seed dispersal [3]. *Simarouba* Kernels were obtained from their seeds after removing outer hard shells by manual dehulling. Severen [13] has also used hand shelled *Simarouba* Kernels in the study and reported difficulty in separation of Kernels from hulls due to their similar densities and brittle nature of hulls. *Simarouba* seeds used had Kernel to hull ratio in the range of 35-37; 63-65. In the earlier reports, workers have reported significant variation in the Kernel content which was dependant on the source/ location of *Simarouba* seeds. Chikara *et al.*, [14] have reported significant variation in Kernel percentage, which ranged from 34.1 to 43.6g/100g, with an average of 40.1g/100g. Earlier Rao and Lakshminarayana [15] have noticed 40g/100g Kernel in *Simarouba* seeds of sub humid region. Severen [13] has reported 30g/100g Kernel and 70g/100g shell in *Simarouba* seeds of South America origin. Dash *et al.*, [16] conducted studies to investigate the physical properties of *S. glauca* L. fruit and Kernel namely dimensions, 1000 unit mass, fruit part fraction, arithmetic mean diameter, geometric mean diameter, surface area, sphericity, aspect, ratio, bulk density, true density, porosity and angle of repose. Moisture content in *Simarouba* Kernel was found to be 4.7g/100g.

It is pollinated by bees. The ripen fruits are eaten by birds and play an important role in seed

dispersal [ICRAF Agroforestry Tree Database <http://ecocrop.fao.org/ecocrop/srv/en/cropView?id=9785>. 2007]. According to Joshi and Joshi [3], due to segregation of staminate (male), and andromonoecious and pistillate (female) plants in natural population the female trees are heavy bearers than male plants. They speculated that it is necessary to plants more number of high yielding females or these male plants can be transformed into female plants by top pruning and *in situ* crown grafting. Thus, unwanted male plants can successfully be transformed into high yielding female, as well as low yielder females can be transformed into high yielder.

## II. Ethenobotanical and pharmaceutical aspects

Online database on *Simarouba* created by Lele [<http://www.svlele.com/Simarouba.htm>. 2010] indicate the leaves and bark of *Simarouba* have long been used as a natural medicine in tropics. *Simarouba* was first imported into France from Guyana in 1713 as a remedy for dysentery. French explorers noticed that the indigenous Indian tribes in the Guyana rainforest used *Simarouba* bark as an effective treatment for malaria and dysentery. Other indigenous tribe throughout the South uses bark for fevers, malaria and dysentery as a haemostatic agent to stop bleeding and as a tonic. Further Lele [<http://www.svlele.com/Simarouba.htm>. 2010] summarized the long history of *S. glauca* in herbal medicine in many other countries. In Cuba, where it is called gavilan, an infusion of the Leaves or bark is considered to be astringent and used as a digestion and menstrual stimulant and an antiparasitic remedy. It is taken internally for diarrhea, dysentery, malaria and colitis. It is used externally for wounds and sores. In Belize the tree is called negrito or dysentery bark. There the bark (and occasionally the root) is boiled in water to yield a powerful astringent and tonic used to wash skin sores and to treat dysentery, diarrhea, stomach and bowel disorders, hemorrhages and internal bleeding. In Brazil it is

employed much the same way against Fever, malaria, diarrhea, dysentery, intestinal parasites indigestion and anemia. In Brazilian herbal medicine *Simarouba* bark has long been the most highly recommended (and most effective) natural remedy against chronic and acute dysentery. Bark and leaf of *Simarouba* contain triterpenes useful in curing amoebiasis, diarrhea and malaria. Joshi and Joshi [3] speculated that the chemicals present in leaf, fruit, pulp and seed of *S. glauca* are known to possess the medicinal properties such as analgesic, antimicrobial, antiviral, astringent, emmenagogue, stomachic, tonic, vermifuge.

*Simarouba* extract is used for reducing patchy skin pigmentation (US Patent Issued on October 14, 1997). *Simarouba* is subject of one US Patent, whereby its water extract was found to increase skin keratinocyte differentiation and to improve skin hydration and moisturization [7]. The seeds extracted in alcohol are used against snake bites. An infusion of the bark is used against malaria, rheumatism, shingles and fever. [[www.tropilab.com/Simarouba.html](http://www.tropilab.com/Simarouba.html)] Similar properties have been described in other Latin American [17], the antiprotozoal [18] and antibacterial [19] activities. Studies of Valdés *et al.*, [20], revealed a strong inhibitory activity against all protozoa tested, but without selectivity. In addition, Cuban folk medicine information also shows several other medicinal uses for these plants, including antihelminthic, antidyseric and antihypertic action [17]. They are useful in curing amoebiasis, gastritis, ulcers in alimentary systems, chikun gunya and malaria. A febrifuge made by extracting the astringent juice of bark used as remedy for diarrhea. Panhwar

[<http://farzanapanhwar.blogspot.com/2007/08/simarouba-galauca-a-new-forest-plant-in.html>. 2007]. Caceres *et al.*, [19] and Lidia *et al.*, [21], reported that the extract of *S. glauca* have been used in Guatemala for the treatment of gastrointestinal disorders. Assendift *et al.*, [22] isolated glaucarubin, a crystalline glycoside from

*S.glauca* and found to have amebicidal properties in vitro and in experimental animal amebiasis were evaluated in the treatment of human infection. They observed that cure rate was in the order of 70 percent following treatment by mouth for 10 days with daily doses of 5 mg/kg (maximum daily dose 300 mg) with exception of vomiting in 2 patients and a transitory decrease in leucocyte count in another, the drug was well tolerated in 113 patients.

*Simarouba glauca* contains Glaucarubin having antiamebic property [23, 24, 25, 26]. Franssen [18] studied the antiparasmodial and cytotoxic effects of four plants commonly used in Guatemalan folk medicine against malaria. They noticed that the Methanol extracts of *S.glauca* DC. *Sansevieria guineensis* willd, *Croton guatemalensis* Lottsy and *Neurolaena lobata* (L) R.Br. significantly reduced parasitemias in *Plasmodium bergheri* infected mice. They screened Dichloromethane fractions for their cytotoxicities on *Artemia salina* (brine shrimp) larvae and 50% inhibitory concentrations were determined for *Plasmodium falciparum* in *in vitro* cultures. They concluded that both chloroquine susceptible and resistant strains of *P. falciparum* were significantly inhibited by these extracts of all dichloromethane extracts, only the *S. glauca* cortex extract was considered to be toxic to nauplii of *A. salina* in the brine shrimp test. The quassinoids, glaucarubin along with glaucarubinone and glaucarubol from the seeds of *S.glauca* showed promising activity against *Plasmodial falciparum* in culture [18, 27]. Glaucarubin was shown to have amoebicidal properties by both an *in vitro* method and in experimental animals [28, 29]. Nurhanan *et al.*, [30] isolated alkaloids with high toxicity and quassinoids with antimalarial and cytotoxic characteristics from *Simarouba*. The antiplasmodial and cytotoxic properties of quassinoids are both linked to protein synthesis inhibition [31], and it is likely that parasite and host cell ribosomes are too similar to allow for development of selective inhibitors [32]. Because

some quassinoids have shown greater selectivity against *P. falciparum* than against cellular lines, chemical derivation has attracted much attention as supplying potential leads for drug design [33]. Based on eurycomanone structure, a monoacylated derivative with reduced toxicity and potent inhibitory activity of chloroquine resistant *P. falciparum* strain was synthesized by Chan *et al.*, [34]. The results of Valdés *et al.*, [20] strongly supported the view that the *S. glauca* extract should not become a priority for further follow up, because all the observed activities are considered as non specific. They also noticed that the activity obtained against *Microsporium canis* ( $Ic_{50} = 2 \mu\text{g/ml}$ ) was likely to be related to non specificity. Etanolic extracts of three plants were used in Cuba as antipyretic and as antimalarial (*Simarouba glauca*, *Melaleuca leucadendron* and *Artemisia absinthium*) were found active *in vitro* against *Plasmodium falciparum* and marginally active *in vivo* against *Plasmodium berghel* [35] *S. glauca* shows acridal activity [36, 37].

Several quassinoid from *S. glauca* seed have exhibited cytotoxic activity *in vitro* against KB cells (human oral epidermoid carcinoma), including glaucarubin, glaucarubinone, glaucarubol and glaucarubolone [38, 39]. The esters of glaucarubolone, aianthinone and glaucarubinone, exhibited significant activity *in vivo* in the P388 lymphocytic leukemia model [39, 40]. The chloroform soluble extract of *S. glauca* exhibited significant cytotoxicity against several human cancer cell lines [41, 42].

Quassinoids a class of chemicals commonly found in members of family *Simaroubaceae*, are toxic to brine shrimp [43], strongly antiplasmodial [44] and strongly toxic to mice [45] for this type of compound, toxicity in the brine shrimp test (BST) is often used as a tool for biologically guided fractionation of extracts [43]. Quassinoids led to isolation and structural determination of the new quassinoids 2-acetylglaucarubine and 13, 18- dehydro glaucarubinone. The previously known 2- acetyl

glauucarubinone and glaucarubinone were also obtained. The new quassinoid 2 was found significantly to inhibit growth of murine lymphocytic leukemia [38]. The polar fractions 3, 5 and 6 are devoid of the quassinoids listed above (33 r) Fig. No.1 but they contain other more polar quassinoids including quassinoid glycosides [46]. One of the nonglycosides from the polar fractions, bruceine D, had antiamoebic activity comparable to that of bruceines B and C, whereas the glucoside, yadanzioside F, was much less active. The antiamoebic activity of the polar fractions is thus presumably due to the presence of polar quassinoids. However, the position of Methyleneoxy Bridge does not seem to affect biological activity significantly. Quassinoids of both types have antimalarial [47] and antileukemic [48] activities.

### III. Other Uses

#### A. Source of biodiesel and biofuel

Bio-fuel is produced either from renewable vegetable oils both edible and non edible. Bio-fuels and hydrogen have attracted the attention as the fastest growing programs in energy efficiency and renewable energy programs. There have been efforts in developing and advancing technology for biomass energy application such as biofuels, biomass power for electricity and bio-products that could replace petroleum based fuels [Shyam [http://www.fbae.org/2009/FBAE/website/our-position-biofuel\\_potential-future-crop-of-farmers.html](http://www.fbae.org/2009/FBAE/website/our-position-biofuel_potential-future-crop-of-farmers.html) 2009]. A few more oils are to be explored to meet the huge demand for biodiesel. *Simarouba* seeds are considered economically important as they contain 60-70 % oil that can be easily refined, bleached, deodorized and fractionated. It is suitable for edible and non edible purposes [1, Joshi and Joshi, <http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf>].

One such oil could be *S. glauca* which is obtained from seeds of *Simarouba* tree commonly known as paradise tree [49]. *S. glauca*

is considered for biofuel production [50]. Joshi and Joshi [<http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf>] reviewed the application of *S. glauca* seed oil and pulp. They speculated that filtered crude oil can be used to blend with diesel @ 5-10 % while the surplus oil produced can be subjected to transesterification to manufacture biodiesel, a 100 % substitute for diesel (1000-2000 kg / ha/ yr.). The sugar rich fruit pulp can be used in the manufacture of ethanol (800-1000 liters/ ha /yr.). Semi sweet fruit pulp, containing 11-12 % sugars is eaten and is well suited for fermentation or beverage industry [51]. Seeds of *S. glauca* are engine tested for alternative biodiesel-feedstock [52]. Biodiesel of *Simarouba glauca* is a simple biodegradable, non toxic and essentially free from sulphur and aromatics and can be used in compression – ignition quality even when blended in petroleum diesel (Shyam [http://www.fbae.org/2009/FBAE/website/our-position-biofuel\\_potential-future-crop-of-farmers.html](http://www.fbae.org/2009/FBAE/website/our-position-biofuel_potential-future-crop-of-farmers.html) 2009). The oil cake, fruit pulp, leaf litter and unwanted wood can be used to generate biogas. The shell and waste wood can be used in thermal power generation. The lignocellulose contained in the huge amount of biomass produced (about 15 tones/ ha /yr) can be used as feedstock for manufacturing second generation biofuels [Joshi and Joshi <http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf> 2007]. The shells of endocarp can be used in activated charcoal industry. They can also used to heat boilers as they possess high calorific value. According to Joshi and Joshi [<http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf>] the fruit pulp contains about 11% sugars. The pulp can be used in preparation of squash, beverage and jam which is very well accepted because of their attractive natural color, flavor and good taste. The fruits can be a source

of natural colorant. *Simarouba* seed contains 35-40% kernel. *Simarouba* fat is primarily used for edible purposes and compares favorably with other fats [13].

### B. Source of vegetable oil

*Simarouba* forms an important source of edible oil for various South and Central American Countries and is widely grown in countries like Costa Rica, El-Salvador, Honduras Cuba, Nicaragua, Mexico, Haiti, Jamaica [7] From 1950 onwards, in El-Salvador and other Central American Countries the oil marketed for edible purposes under the trade name manteca vegetal "nieve" and oil is well accepted [3]. The use of *S. glauca* as a vegetable oil crop was first considered near the end 1939. The oil extracted from seeds in existing oil mills and processed by adopting conventional methods [52, Joshi and Joshi, <http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf>]. Monseur and Motte [40] reported that the seeds of *S. glauca* are rich in edible fat (nearly 60 % W/W) that has been used for cooking in tropical countries. In other developing countries, also it can be used to manufacture vanaspati, vegetable butter and margarine. The Refined, Bleached and Deodorized (RBD) oil is further fractionated to separate the liquid and solid fractions. The liquid fraction with very high oleic acid content (about 85 %) is comparable to olive oil in its chemical composition. This oil is free from bad cholesterol (free fatty acids 0.06%). The solid fraction rich in steric acid and palmitic acids can be used as coco-butter substitutes (CBS) or coco-butter extenders in confectionary and bakery industries [54]. The palmito stearin fraction is also useful in preparation of ice cream and mayonnaise [Joshi and Joshi, <http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf>]. Fruit is good source of vegetable oil which is rich source of fat soluble vitamins like A and E [Panhwar <http://farzanapanhwar.blogspot.com>

[/2007/08/simarouba-galauca-a-new-forest-plant-in.html](http://farzanapanhwar.blogspot.com/2007/08/simarouba-galauca-a-new-forest-plant-in.html). 2007]. *Simarouba* oil is also used in industrial manufacture of soap, lubricant, paint; polishes and pharmaceuticals etc. shells (endocarp) are used in hard board industry [7]. The physical properties of oilseed are important in designing and fabricating particular equipments and structures for handling, transporting, processing, and storage and also for assessing the behavior of product quality [55, 56]. Physical properties of *Simarouba* fruit and kernel are essential to design equipments for decoration, drying, cleaning, grading, storage and oil extraction. Though literatures are available on *Simarouba* plants and its oil characteristics [57, 58] no study has been done on its physical properties.

### C. Timber

The main trunk of ten years old tree produces 5-10 cubic feet of wood. The wood is light attractively grained, moderately strong generally less preferred by wood eating insects hence useful in making yolk for oxen, light furniture, toys ,packing material, pulp for paper industry and match boxes. Waste wood is good fuel [Joshi and Joshi; <http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf>], [Panhwar, [<http://farzanapanhwar.blogspot.com/2007/08/simarouba-galauca-a-new-forest-plant-in.html>. 2007].

### D. Phytoremediation

The paradise tree also improves soil health. It is used as soil and watershed conservation also attracts more rain and helps to control soil erosion. The tree acts as wind breaker Panhwar, [<http://farzanapanhwar.blogspot.com/2007/08/simarouba-galauca-a-new-forest-plant-in.html>. 2007]. The tree forms a well developed root system and dense evergreen canopy that efficiently checks soil erosion supports soil microbial life and improves groundwater position. Besides converting solar energy into biochemical energy all round the year, it checks overheating of soil surface all through the year

and particularly during summer. Large scale planting in wastelands facilitates wasteland reclamation, converts the accumulated atmospheric carbon dioxide into oxygen and contributes to the reduction of green house effect or global warming [Lele [\[http://www.svlele.com/Simarouba.htm](http://www.svlele.com/Simarouba.htm). 2010]. in general soil adjacent to female *S. amara* trees had higher extractable phosphorus levels than soil adjacent to male trees or the control samples. This indicates that not only does *S. amara* influence soil  $P^H$  but that tree gender has an additional influence [59]. Neelavathi *et al.*, [60], studied the removal of toxic  $C_r$  (VI) in aqueous medium using activated carbon adsorbents prepared from *s. glauca* seed shells. They observed that the removal of  $C_r$  (VI) around 97% with 1:2 impregnated activated carbon at  $P^H$  3.0 where as other adsorbent showed much lower activities. *Simarouba* did not survive to the end of the study under high flood treatment. The moist soil conditions simulated by the low flood treatment resulted in greater growth in all species compared to soil inundation under high flood, except for the most flood- tolerant (*Annona*, *Morella*, *Salix*) [61].

#### E. Manure

The oil cake being rich in nitrogen (8%), phosphorus (1.1%) and potash (1.2%) is good organic manure [Lele, <http://www.svlele.com/Simarouba.htm>. 2010]. According to Govindaraju *et al.*, [7] *Simarouba* meal contained high concentration of calcium (143.03 mg /100 g) and sodium (78.93 mg/100g). Whereas the concentration of iron and magnesium was low at 11.1 and 7.5 mg /100g respectively. Concentrations of heavy metals in *Simarouba* meal was found to be below detectable level; cadmium <0.1 ppm, lead <0.5 ppm and arsenic 0.02 ppm . The diurnal loss and recovery of  $K_{leaf}$  in *S. glauca* appears to proceed without the significant hysteresis expected for a relatively slow refilling process [62]. Organic matter content was 17 percent higher adjacent to trees (15.8%) compared to control samples

(13.5%) [59]. *Simarouba* press cake is being utilized as an organic fertilizer and used. The values of press cake may be expressed as follows N-7.7%, P-1.07% and K-1.24%. There are also traces of Ca, Mg, Na. Being bound in organic matter the nitrogen release is gradual a factor which is favorable to most crops growing in the high rainfall areas of tropics. The use of *Simarouba* press cake as a fertilizer has given good results on coffee, sugarcane, cotton and corn [53]. The fruit pulp may be used in the production of vermicompost which is of excellent quality. Leaf litter makes good manure [7]. Leaf litter (about 20kg/tree/yr.) makes good manure, improving the fertility status of soil [Joshi and Joshi, <http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf>]. Deoiled meal cake of *Simarouba* has not been the subject of detailed scientific study. Few researchers have reported high protein content (50-55%) in deoiled meal cake of *Simarouba* [63, 51, 14]. Severen [13] reported presence of bitter principle, related to quassine in residual cake of *Simarouba* which was found toxic to livestock. Vaughan [64] also reported toxicity of *Simarouba* meal. The cake obtained from the extraction for the oil contains proteins used for cattle feed after the removal of toxic and bitter constituents [40]. Protein rich *Simarouba* meal is presently disposed off as manure [14]. Coley *et al.*, [65] noticed that root exudates affect herbivory or microbially mediated mineralization of organic material. Bawa and Opler [66] noticed that the presence of secondary compounds in the rooting zone affect microbial populations and alter plant microbe competition for soil nutrients. They observed that organism specific defense compounds may favor beneficial microbial associations such as vesicular arbuscular mycorrhizae and increase in P uptake. The ash of shells of endocarp can put back to soil to enrich the potash content. The smooth ash blended with a little soap can be used in manufacture of dish washing powder [Joshi and Joshi; <http://ageconsearch.umn.edu/>

bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf]. Being highly productive, its cultivation not only gives good results to the farmers but also encourages the development of several industries and thus promoting better employment opportunities [Gowda, B. Org/production\_Simarouba.html 2008].

#### IV. Physiological studies

Effect of growth regulators on four month stored seeds of *S. glauca* (*Quassia Simarouba*) was studied by Radhakrishnan and Renganayaki [67], to improve germination and vigour potential. They were treated the seeds with 2 concentrations of IAA, IBA and GA<sub>3</sub> (200 and 400 ppm), they noticed that the beneficial effect of IAA at 200 ppm over the rest with 36.33 % germination as against 11.67 % in control 38 days after sowing. They reported that the improvement of vigour by IAA at 200 ppm was 4 fold higher than the control. The root length and root biomass were increased significantly by GA<sub>3</sub> treatment. According to Kureel *et al.*, [www.novodboard.com, 2009], pretreatment is not necessary since it does not have any dormancy. The germination of normal seeds takes 20-30 days. However, the scarification hastens it at least 10-12 days earlier than normal. Scarification is done by soaking seeds in cold water over night or hot water (20 minutes or partial breaking of seed coat). The seeds are germinated in sand at fluctuating 35-45°C and 12 hours light and other dark condition.

Mott and Franks [68] speculated that such uncoupling between stomatal closure and bulk leaf water status suggest that the pressure-volume relation for guard cells is different to that of the bulk leaf, or that guard cells are somewhat hydraulically isolated from the rest of the leaf. These conclusions are supported by evidence for the coordination between stomata where as bulk leaf conditions remain unchanged. Brodribb *et al.*, [69] noticed that the leaf cells lose turgor at a water potential which induced 99% stomatal closure. Thus in species *S. glauca* most of the

stomatal response to leaf water potential ( $\psi_L$ ) occurs as mesophyll cell turgor declines. According to Brodribb and Holbrook [70] mechanism aside, it remains to explain the advantage to leaves in producing a vascular system that is vulnerable to cavitation during normal daily function. They speculated that in case of *S. glauca* probably lies in the relationship between the guard cell environment and the evaporative environment. The high hydraulic conductivity of *S. glauca* leaves means that the guard cell water potential is likely to be dominated by the water potential of the upstream xylem and soil. As a result, even large changes in the evaporative environment of the leaf will impact minimally on the water potential of the guard cells and this may impede the responsiveness of stomata to changes in evaporation flux. It is possible then that a reversible loss of hydraulic conductivity in the leaf may be an adaptive means of amplifying the evaporative demand signal to the stomata in order to expedite a stomatal response. The major benefit of this would be protection of upstream xylem; a realistic motivation considering that the petiole xylem in *S. glauca* is only slightly more resistant to cavitation than the leaf [69] and may be more difficult to repair. One of the evergreen species *Simarouba glauca* produced relatively short-lived leaves that maintained high hydraulic conductance year round by periodic flushing [70].

Oberbauer *et al.*, [71] compared light environment, leaf physiological characteristics and growth for forest grown samplings of three species of tropical trees with known life histories. They tested the species included *Lecythis ampla*, a species tolerant of understory conditions, *Pithecellobium elegans*, a species found in relatively bright sites and *S. amara*, a fast growing, light demanding species. They noticed that with respect to similar light regimes the species differed markedly in leaf area and gas exchange. Leaf areas of *Lecythis* samplings were five and ten fold greater than *Simarouba* and



*Pithecellobium* samplings, respectively. Light saturated leaf photosynthesis and leaf dark respiration rates of *Lecythis* were about half those of *Simarouba* while rates of *Pithecellobium* were intermediate. They concluded that *Lecythis* had the highest leaf photosynthesis at understory diffuse light with the strongest correlations between sapling performances and diffuse light. Ryan et al., [72] measured CO<sub>2</sub> efflux from stems of 2 tropical wet forest trees, both found in the canopy, but with very different growth habits. The species were *S.amara* a fast growing species associated with gaps in old growth forest and abundant in secondary forest and *Minquartia guianensis*, a slow growing species tolerant to low light conditions in old growth forest. They reported that per unit of bole surface, CO<sub>2</sub> efflux averaged 1.24 μ mol/m<sup>2</sup>s<sup>-1</sup> for *Simarouba* and 0.83 μ mol/m<sup>2</sup>s<sup>-1</sup> for *Minquartia* CO<sub>2</sub> efflux was highly correlated with annual wood production ( $r^2=0.65$ ), but only weekly correlated with stem diameter ( $r^2=0.22$ ). CO<sub>2</sub> efflux from stems of two wet forest trees varied seven fold, but was only related to stem diameter. Their results showed that partitioning CO<sub>2</sub> efflux into the functional components of construction and maintenance respiration can explain much of the variability in CO<sub>2</sub> efflux from stems of wet forest trees. CO<sub>2</sub> efflux was highly correlated with annual wood production, and estimated maintenance respiration was linearly related to sapwood volume.

## V. Phytochemical Constituents

### A. Seed

In the previous work the seeds of *S. glauca* have afforded quassinoids [73, 38, 74, 54] and an alkaloid 8- hydroxyl canthin-6-one [74]. In earlier studies, Rao and Lakshminarayana [63]; Rath et al., [51] and Chikara et al., [14] have reported oil content of *Simarouba* in excess of 60g/100g. Whereas Severan [13] observed that *Simarouba* Kernel contained fat in the range of 55-65g/100g. Protein content in the *Simarouba* Kernels was 18.2g/100g which increased to

47.7g/100g in defatted meal of *Simarouba*. Meal of *Simarouba* contained residual fat of 1.1g/100g [7]. Its seeds contain 50-65% oil that can be extracted by conventional methods. Each well grown tree yields 15-30 kg nutlets per year equivalent to 2.5-5 kg oil this amounts to 1-2 t oil per ha per year and about the same quantity of oil cake [75] scanty scientific literature available on *Simarouba*, mainly deals with composition and characteristics of its fat. Jeyarani and Reddy [54] reported that the seeds contain 40% Kernels and the kernels contain 60% fat, which is edible. The odorless, greenish yellow fat melts at 26.4 c, has an iodine value of 52.6 and a saponification value of 190.5, Fatty acid composition of *Simarouba* fat has been investigated by several researchers [63, 51, 14] and major components are oleic (52-54%), stearic (27-33%) and palmitic(11-12%). Further it has been reported that characteristics of the fat and fatty acid composition of Indian origin do not significantly differ from those reported from seeds of other countries [63, 51] considering the high fat content in the kernels and moderate iodine value and high content of oleic and steric acids, the fat has good potential for use as edible fat or for blending with vanaspati or for use as cocoa butter (CB) substitute or extender. *Simarouba glauca* is rich source of fat having a melting point of about 29<sup>0</sup>C and consisting of palmitic (12.5%) oleic (56%) and stearic (27%) as major fatty acids. It consists of about 30% of symmetrical monosaturated type triacylglycerols and appears to be a good source of fat for preparation of cocoa butter (CB) extender [54]. They revealed that the stearic fraction obtained from *S. glauca* fat after removal of about 65% oleic fraction is suitable for use in chocolate products as CB extender. The odorless, greenish yellow fat melts at 26.4<sup>0</sup>C and has an iodine value of 52.6 and saponification value 190.5 [63]. Rath et al., [51] and Severan [13] have reported slightly higher protein content of 50g/100g in meal cake of *Simarouba*. On the other hand Chikara et al., [14] during the study of *Simarouba* from different

sources has reported range of protein values (45.6-56.8g/100g; average, 51.8g /100g) in their deoiled meal cake. Similarly crude fibre content of *Simarouba* Kernel (8.1g/100g) increased to 11.8g/100g in its deoiled meal. In order to exploit the protein rich (47.7g/100g) *Simarouba* meal in food/feed, Govindraju *et al.*, [7] conducted studies on its chemical composition with emphasis on protein characteristics and toxic constituents. They noticed that *Simarouba* meal contained high calcium (143mg/100g) and sodium (79mg/100g) while saponins with triterpenoid aglycone (3.7g/100g), alkaloids (1.01g/100g), phenolics (0.95g/100g) and phytic acid (0.73g/100g) as the major toxic constituents identified in *Simarouba* meal. Their results of TLC and HPLC studies indicated that among different fractions of *Simarouba* saponins, one dominant fraction accounted for about 28% proteins of *Simarouba* recorded high *in vitro* digestibility (88%). While SDS-PAGE studies revealed four major protein bands in molecular weight ranges of 20-24, 36-45, and 55-66 k Da. They observed that apart from glutamic acid (23.43g/100g protein) and arginine (10.75g/100g protein), *Simarouba* protein contained high essential amino acids like leucine (7.76g/100g protein), lysine (5.62g/100g protein) and valine (6.12g/100g protein). Finally they concluded that among nutritional indices, *Simarouba* meal recorded a good EAA Index (75.02), C-PER (1.90) and PDCAAS (1.0 Adult group).

Amino acid composition of *Simarouba* meal, along with that of reference Soy protein (FAO/WHO, 1958) is given in Table No. 1. Result of amino acid analysis indicated that glutamic acid (23.43g/100g protein), arginine (10.75g/100g protein) and aspartic acid (10.50g/100g protein) are the major amino acids in *Simarouba* meal, which is typical of oil seed proteins. Among essential amino acids, *Simarouba* meal contained greater levels of leucine (7.76g/100g protein), lysine (5.62g/100g protein) and valine (5.62g/100g protein), when compared with EAO/WHO (1985) recommended

reference soy protein *Simarouba* meal was found to be deficient in sulphur containing amino acids, methionine and cysteine. [7].

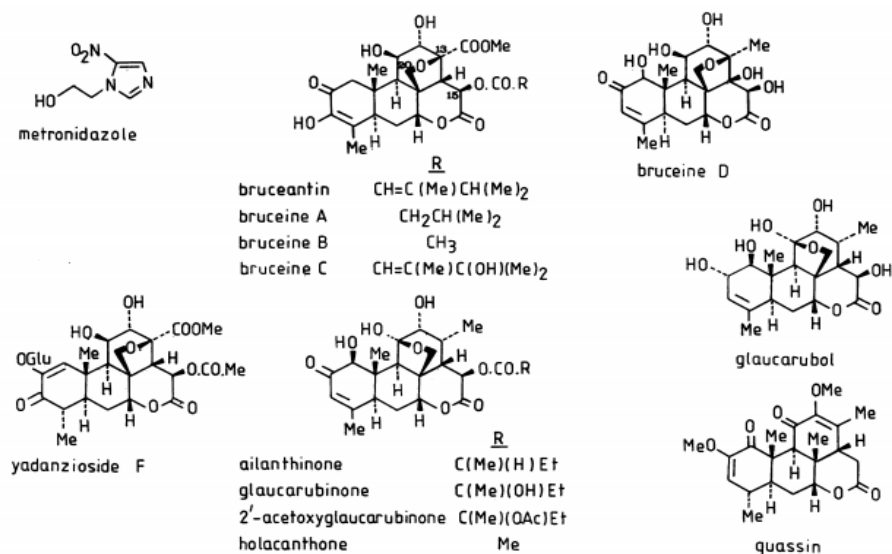
**Table No. 1.** Comparison of amino acid composition of *Simarouba* meal and soy protein.

Amino acid (g/100 g protein)	Simarouba meal	Soy bean (FAO/WHO Reference Protein)
Asp	10.5	11.7
Glu	23.43	18.7
Ser	3.99	5.1
Gly	4.94	4.2
His	2.54	2.5
Arg	10.75	7.2
Thr	2.9	3.9
Ala	4.71	4.3
Pro	3.39	5.5
Tyr	2.16	3.1
Val	6.12	4.8
Met	0.38	1.3
Cys	0.4	1.3
Iso	0.62	4.5
Leu	7.76	7.8
Phe	5.86	4.9
Lys	5.62	6.4
Trv <sup>a</sup>	1.35	1.3

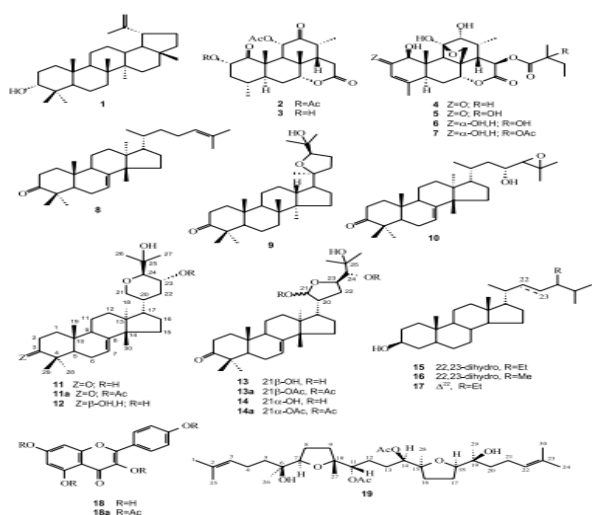
<sup>a</sup> Estimated by ninhydrin method.

Source- Govindaraju *et al.*, (2009)

Polar quassinoids have not yet been detected in *Simarouba amara* although the related species *S. glauca* produces 15-O glucocides of glaucarubol and glaucarubolone in the seeds [45]. Quassinoids are bitter terpenoids with a polycyclic skeleton that a modified in various ways so that, among the simaroubaceae, a number of differences between quassinoids found in *Brueca javanica* and those present in *S. amara*



**Fig. No. 1.** Structure of metronidazole and the quassinoids.



**Fig. No. 2.** Structure for compounds isolated from *s. versicolor* and derivatives

is the location of the methyleneoxy bridge, the oxygen is attached to C-11 in *S. amara* and the carbon is attached to C-8 in both. Arriaga *et al.*, [76], isolated quassinoids from *Simarouba versicolor* roots, stems and fruits these are quassinoids (3, 5-7), triterpenoids (8-14), a mixture of steroids (15-17), the flavonoid kaempferol (18) and the squalene derivative 11,14-diacetoxy-7,10; 15,18-diepoxy-6,19-dihydroxy-6,7,10,11,14,15,18,19-

octahydro squalene (19). Epilupeol (1), four quassinoids (2-5) and b-sitosterol (15) were previously isolated from the leaves and stems of a specimen of *Simarouba versicolor* collected in the State of Paraná, Brasil [77]. Glaucarubinone (5) [78,79, 80] glaucarubin (6) [81, 80] were previously obtained from *Simarouba glauca* [82] 2'-Acetylglaucarubin (7) [79] and tirucalla-7,24-dien-3-one (8) [78, 83] were reported as bioproducts from *S. amara* [82]. Figure No. 2

### B. Antinutritional composition

Literature survey on toxic and antinutritional factors of *Simarouba* meal indicated that very scanty literature pertaining to identification and estimation of different toxic and anti nutritional factors in *Simarouba* meal is available , systematic study was conducted by Severen [13], Vaughan,[64] Rath *et al.*,[51]. Studies of Govindaraju *et al.*,[7] indicated that deoiled meal of *S. glauca* is rich source of protein (47.7 g/100g) with high solubility (92%), *in vitro* protein digestibility (88%)and amino acid based computed nutritional indices, They revealed after identification and estimation of toxic constituents that *Simarouba* meal required detoxification from saponin(0.95g/100g), alkaloid (1.01g/100g),

phenolics (0.95g/100g) and phytic acid (0.73g/100g) before its usage potential could be utilized in feed/ food formulations. Govindraju *et al.*, [7], further reported that proteins of *Simarouba* meal show relatively *in vitro* digestibility of 88%. They speculated that high *in vitro* protein digestibility in *Simarouba* meal could be attributed to lower tannin levels, which inhibit the trypsin digestion. Further *Simarouba* protein might not be bound to minor constituents by glycosidic or ester linkages making it easily digestible. Considering the high protein content of *Simarouba* meal (~ 48g/100g) investigations carried out by Govindraju *et al.*, [7] on its different quality characteristics in order to determine the potential of its usage as feed or food. Their result indicates that protein solubility profile of *Simarouba* meal as a function of PHC (2-10) followed 'U' pattern which is typical of oilseed proteins. *Simarouba* seed protein had solubility minima (23.7%) and iso-electric precipitation at PH 4.0 on the other hand, the *Simarouba* proteins showed maximum solubility (92%) at PH 9.0. Typical protein solubility profiles with similar solubility values were reported by wang *et al.*, [84]; Rakshit and Bhagya [85] and Vinay and Sindhukanya [86] for rice bran, *Jatropha* and *Karanja* Proteins respectively.

To identify different anti nutritional factors in *Simarouba* meal related to quassine. Studies of Govindaraju *et al.*, [7] indicated that of the methanolic extract of *Simarouba* meal gave purple mauve color with anisaldehyde in sulphuric acid, for the presence of saponins. They had purified saponins; the methanolic extract of *Simarouba* meal was passed through ion exchange column, using di-ion HP20 resin colorimetric determination indicated that *Simarouba* meal contained 3.7g/100g total saponins. They subjected Saponins from *Simarouba* meal, to Lieberman-Burchard reaction, using acetic acid and sulphuric acid gave pink color. This indicated that aglycone portion of saponin is triterpenoid type. They

speculated that saponins are amphiphilic compounds, in which sugars are linked to non-polar group (Sapogenin), which may be either a sterol or a triterpene. Their studies on haemolytic activity indicated potent haemolysis in samples of *Simarouba* saponins. Samples of *Simarouba* saponins in 0.9% saline they reacted with erythrocytes and haemoglobin released were measured at 540 nm. They showed that crude saponin of *Simarouba* was comparable to haemolysis; where as purified saponin of *Simarouba* was much more potent in haemolysis, showing HD50 value at 0.5.-0.60mg/ml compared to HD50 value of soy saponin at 1.20-1.40 mg/ ml.

### C. Bark Constituents

*Simarouba* bark is obtained as long pieces from 4-12 cm wide and 2-5 mm thick, folded lengthwise, light flexible, tenacious, very fibrous, externally of a light brownish yellow color, rough warty and marked with transverse ridges, internally of a pale yellow. It is without odour and of a bitter taste (Remington and Wood, <http://www.henriettesherbal.com/electric/usdisp/simarouba.html>. 1918). Gilling [87] isolated from *Simarouba* bark a crystalline bitter substance, simaroubin, to which he assigned a formula  $C_{22}H_{30}O_9$ . Some thirty years later, Glemser and Ott [88], examined the crystalline constituents of the bark of *S. amara*. They isolated simaroubin and confirmed the formula  $C_{22}H_{30}O_9$  and another of undetermined composition were also obtained [73]. It is resinous matter, a volatile oil having the odour of benzoin, malic acid, gallic acid in very minute proportion, an ammonical salt calcium malate and oxalate, some mineral salts, ferric oxide, silica, ulmin and lignin. Remington and Wood, [<http://www.Henriettesherbal.com/electric/usdisp/simarouba.html>. 1918] noticed that *Simarouba* bark is used as a bitter and astringent in chronic dysentery. For the latter purpose, a decoction is prepared (1 in 20), often with an equal quantity of Cinnamon bark. Dose-1-2g (15-30 grains). Studies of Ham *et al.*, [73] led to the

isolation of crystalline compound from *S. glauca*, which has been designated glaucarubin. Rivero-Cruz *et al.*, [89] investigated bioassay guided phytochemicals of *S. glauca* stem extract, using the KB cell lines as a monitor, led to the isolation and identification of canthin-6-one(1),2-methoxy canthin-6-one(2),9- methoxycanthin-6- one(3),2-hydroxy canthin-6-one (4), melianodiol(7 )and 14-deacetylerylene(8) as cytotoxic principles together with two further canthin-6- one alkaloid derivatives. 4-5 dimethoxy canthin-6-one (5) and 4-5 dihydroxy canthin-6-one(6), two coumarins, scopoletin(9)and fraxidin(10) and two triglycerides, triolein(11)andtrilinolein (12) as inactive constituents. After their purification six canthin-6-one alkaloids (1-6), melianodiol (7), 14- deacetylerylene (8), scopoletin(9),fraxidin (10) ,triolein(11)andtrilinolein (12), isolated from twigs of of *S. glauca* were tested for cytotoxicity against a panel of human cell lines .The main plant chemicals in *Simarouba* include – aianthinone, benzoquinone, canthin, dehydro–glaucarubinone , glaucarubine, glaucarubolone, , glaucarubinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol and triucalla [<http://www.RaintreeNutrition.Com> 2010].

## VI. Biotechnology

*Simarouba glauca* (Simaroubaceae), a fast growing multipurpose tree, grows even on marginal lands under water stress conditions [90]. In vitro propagation of *Simarouba glauca* was reported by Rout and Das [91]. Of the four kinds of explants tested, only the shoot and nodal segments allowed somatic embryo induction and development. Absence of plant growth regulators/ cytokinins generally yielded better developmental responses for *S. glauca* [90]. They observed that frequency of somatic embryogenesis from callus cultures derived from immature cotyledon explants of *S. glauca* was highest on solid M.S. medium supplemented with 11.1µM benzyl adenine and 13.42µM NAA was

highest on M.S. solid medium supplemented with 1.11 µM and 13.42 micro m benzyl adenine NAA while after transfer of the somatic embryos into maturation medium containing half strength M.S. medium supplemented with 1.89 µM abscisic acid (ABA) and 2% (W/V) sucrose,20-25% of embryos germinated within 20 days of culture with distinct cotyledon, hypocotyls and radical. *In vitro* shoot multiplication was achieved on MS medium containing 2.5 mg /l BA with 0.1 mg /l NAA and a maximum of 5.83 shoots were produced per nodal explants within 6 weeks of culture [91]. Elongated shoots were rooted on MS medium supplemented with 1.0 mg /l to 1.5 mg /l IBA. Studies of Rout *et al.*, [92], indicated that the rooting occurred between the 12<sup>th</sup> and 15<sup>th</sup> day of culture on MS medium supplemented with 1.0 mg / l IBA while the percentage of rooting was the maximum (82.45%) on medium having 1.0mg/l IBA rooting was inhibited on the devoid of IBA. The number of roots/shoot significantly varied with different concentration of IBA. Roots produced in 1.0mg/l IBA were healthier than that produced in higher concentration of IBA (1.5mg/l to 2.0mg/l). The media containing auxin stimulated the induction of rooting. They achieved induction of rooting in microshoots of *Simarouba glauca* L. within 12-15 days of culture on Murashige and Skoog's [93] medium supplemented with 1.0 mg /l IBA and 3% (W/V) sucrose. They noticed that there was no spontaneous rooting observed without the application of auxin while peroxidase activity was the minimum at induction phase and maximum at the initiation and expression phase grown on medium containing 1.0mg / l IBA. They reported that rooting was associated with selective expression or repression of isoforms of peroxidase during induction, initiation and expression phase which indicates a key role of peroxidase in rooting of microshoots of *S. glauca in vitro*. According to Chwala [94], enzymes which are known as metabolic markers, change during development and differentiation. Rout *et al.*, [92], observed that based on the peroxidase

isozyme analysis at different intervals during the rooting process, the rizogenesis accompanied by the synthesis of certain proteins and enzymes. In the primary (induction) phase, four ethanolic bands (RF=0.20, 0.28, 0.33 and 0.37) and anodic bands having RF values ranging from 0.62 to 0.64 were observed. After 3 days of culture on rooting media three cathodic bands disappeared and two anodic bands reappeared having RF values 0.54 and 0.62. On the 6<sup>th</sup> and 9<sup>th</sup> day of culture, the appearance and disappearance of anodic and cathodic bands were noted. During initiation of rooting four cathodic bands (RF=0.20, 0.28, 0.33 and 0.37) and two thick anodic bands (RF=0.43, 0.48) became visible which might be an additional multiple molecular form of enzyme marker during rhizogenesis. The number and intensity of anionic peroxidases continuously increased during the process of rhizogenesis. They determined the peroxidase activity in microshoots on different treatments during the rooting process. They observed that activity became less apparent in microshoots derived from the media without the growth regulator where as the peroxidase activity was also the minimum at primary (inductive) phase and maximum at secondary (imitative) phase in microshoots grown on medium having 1.0 mg/l IBA. The minimum peroxidase activity was observed between the 0-day and the 9<sup>th</sup> day; maximum activity however, was noted between the 12<sup>th</sup> and 15<sup>th</sup> day. The results of Rout *et al.*, [92], confirmed that during rhizogenesis, peroxidase activity was the minimum in the primary (inductive) phase and maximum at secondary (initiation) phase in relation to auxin treatment.

Tissues from five male plants, five female plants and two andromonoecious plants were obtained from *Simarouba* plantation of the University of Agricultural Sciences, Bangalore were obtained by Joshi *et al.*, [95] *Simarouba* segregates into male, female and andromonoecious during flowering. Since controlling is important. An attempt has been by

Joshi *et al.*, [95], to develop markers for polyamodioecious character in *Simarouba*. They screened nine enzyme systems were screened with four different tissues, four extraction buffers and four electrode buffers. They noticed that the use of Ferret extraction buffer containing ascorbic acid, Tris-Borate-EDTA electrode buffer and flower tissues, five enzyme systems gave good resolution and staining. They studied these enzyme systems using starch gel electrophoresis and no polymorphism was detected. To access variability, they analyzed plants using randomly amplified polymorphic DNA (RAPD). Out of one hundred and fifty decamer primers used. They selected Seventeen decamer primers based on amplification polymorphism. They subjected the binary data obtained to cluster, 2D-PCA and 3D-PCA analysis, which showed five clusters. They speculated that t-test of two primers OPT-7 and OPW-3 could be used to differentiate between males and females. While one primer, OPS-6 gave a characteristics band only in 100% males and not in others.

Studies of Simon *et al.*, [96] initiated to determine useful sex linked RAPD markers in *Simarouba*. They have selected seventy 10-mer operon primers and used to generate consistent, clear amplification products ranging in size from 250 bp to 4.0 kbp. They observed that primer OPD-20 amplified a band of approx. 900 bp which was consistently present in all female individuals tested. Their results showed that RAPD analysis is an effective marker technology with which to develop sex linked markers to enable the elimination of male seedlings.

Sailo and Bagyaraj [97], conducted a glasshouse study to investigate the influence of the *Arbuscular mycorrhizal* (AM) fungus, *Glomus mosseae* and plant growth promoting rhizomicroorganisms (PGPRs). *Bacillus coagulans* and *Azotobacter chroococcum*, alone and in combination, on the growth and nutrition of *S.glauca*. They noticed that Individual inoculation of these organisms significantly

enhanced plant biomass, but biomass was significantly greater with inoculation of *G.mosseae* than with inoculation with *coagulans* further significantly enhanced plant biomass compared with all other treatments. This was also reflected in other parameters studied; plant height, no. of leaves and plant P-content. They also observed that the percent mycorrhizal colonization in the root and spore in the root zone soil were also highest in seedlings inoculated with both *Glomus* and *Bacillus coagulans*.

## VII. Cultivation Practices

### A. Propagation

*S. glauca* propagated from seeds, grafting, air layering, cutting and tissue culture [Joshi and Joshi, <http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20B%20angalore,%20India.pdf>], [1]). According to Panhwar, [<http://arzanapanhwar.blogspot.com/2007/08/simarouba-galauca-a-new-forest-plant-in.html>. 2007], sexual Propagation of *S. glauca* will take 5-8 years while asexual Propagation attains stability of inflorescence types and sexual characteristics within 18 months to 3 years. She noticed that budding and the rooted cuttings were unsuccessful where as the cuttings of 4-6 inches diameter and 8 feet long sticks are inserted at monthly intervals which are found successful in the development of root and shoot system. She also reported that whip and tongue grafting methods are suitable for grafting *S. glauca* during June to August.

### B. Climate and Soil

*S. glauca* is tropical tree established in regions with 250 mm to 2500 mm rainfall and found to grow upto 1000m above sea level [14].It is found to grow luxuriantly up to altitude 0-800 m and in between the temperature 21.5<sup>o</sup>C to 28.6<sup>o</sup>C can tolerate mean maximum monthly temperature up to 45<sup>o</sup>C in all types of well drained soils and with an average annual rainfall of 1,769 mm in central region and 1,833 mm for those in eastern region [1]. It prefers to grow well in soils with 5.5 to 8.5 P<sup>H</sup> [ICRAF Agroforestry Tree Database,

<http://ecocarp.fa.org/ecocrop/srv/en/cropView?id=97852007>]. It is luxuriantly grown in red loams and red lateritic soils with higher productivity [3, 98].

### C. Artificial Regeneration, Seed Maturity and seed collection

According to ICRAF Agroforestry Tree Database [<http://ecocarp.fa.org/ecocrop/srv/en/cropView?id=97852007>] and TNAU[ [http://agritech.tnau.ac.in/bio-fuels/Biofuel\\_paradise.html](http://agritech.tnau.ac.in/bio-fuels/Biofuel_paradise.html) 2008], the *Simarouba* seeds attain physiological maturity, maximum germination capacity and longevity at 11-13 weeks after flowering.. These ripe seeds are available during April and May. April ripe blackish purple berries are considered as good material for its regeneration from seeds. They advised that the time of seed collection discard green fruits which account for poor quality and dark purple colored fruits are collected and pulp must be removed immediately after collection either by hand or in a depulper. Immediately dried in shade for few hours followed by sun drying to reduce the moisture content upto 12-15 % after drying and cleaning, empty, immature, broken or insect damaged seeds are removed. They recommended that Separation of full and empty seeds due to specific gravity differences can be accomplished by liquid flotation techniques.

### D. Storage and viability

Germination of fresh *Simarouba*, seed is 70 to 80 %. The *Simarouba*, seeds have short viability of 2-3 months. Seeds are stored in paper or cloth bags at room temperature for 9- 12 months or at low temperature to retain high viability for several years. [Kureel *et al.*, [www.novodboard.com](http://www.novodboard.com), 2009].

### E. Development of nursery

According to TNAU Web page [[http://agritech.tnau.ac.in/bio-fuels/Biofuel\\_paradise.html](http://agritech.tnau.ac.in/bio-fuels/Biofuel_paradise.html) 2008], *S. glauca* can be easily propagated from seeds. It can also be grown through vegetative propagation techniques like grafting, air layering *etc.* The mature depulped seeds after drying for 2-3 days under

shade are good for raising seedlings. The seeds are sown in a nursery bed or directly in containers. The polybags (15 × 25 cm size) are filled with the nursery mixture (soil: sand: FYM in the ratio of 3:1:1) the single seed is sown in each bag. The seed start germination on 15<sup>th</sup> day after sowing and requires 25 days for complete germination. The germination rate varies between 60-80 % which depends upon local factors. Hence, to ensure higher viability of the seeds, sowing the seeds within six months of collection is highly recommended.

The TNAU database [http://agritech.tnau.ac.in/bio-fuels/Biofuel\_paradise.html. 2008], further enumerates the technique of sowing in mother bed. A raised nursery bed has to be prepared at a size of 10 m × 1m. The soil mixture used to fill the bags consists of garden soil, sand and compost in the ratio 1:1:1. The mixture is thoroughly pulverized and sieved. The bags are watered twice a day. The seeds are generally sown in lines. Usually the lines are made at 10-15 cm apart and the seeds are sown in 3-5 cm apart. The depth of the sowing should be 2-4 times the diameter of seeds, and ensure that the seeds are just covered with soil. The transplanting of seedlings can be done the seedlings are 7-10 cm tall with taproot about 15 cm long (40-50 days after sowing). Pricking out of seedlings is done generally at the stage of 10-22 cm or 15-22 cm. Since, the taproot grows faster than shoot, frequent shifting of seedlings should be done to prevent the roots from striking the ground. Six months old seedlings are ready for out planting. Application of biofertilizers may enhance the quality of seedlings and reduce the nursery period. The novodboard database [www.novodboard.com] indicates that 30-45 days old seedlings are good for planting in the main field, April harvested fruits produces seedlings of one month age by first week of June. This ensures better post monsoon stand in the field. This database also depicts the late planting often inhibits growth due to short rainy season or

aberrant weather condition. Thus to attain this objective, seedlings are raised from previous year stock or through vegetative propagation methods of clonal propagations, grafting and air layering ensuring good results of 70-80% success. The novodboard database also indicated that saplings or grafts are planted in a space interval of 5.0 m (7.0 to 8.0 meters in high rainfall areas) in the pits of 45× 45× 45 cm size half filled with top soil and 2.0 kg compost + 20 g phosphorus+ 20 g nitrogen. Chemical fertilizers like N, P and K may be applied in the proportion of 50 g, 25 g and 25 g respectively during first year of planting in two split doses that is pre monsoon and post monsoon application for good result. *Simarouba* can be cultivated profitably in alley cropping, boundary planting, and bund planting, as plantations or as avenue trees. They can be also planted as dooryard trees [Joshi and Joshi, http://ageconsearch.umn.edu/bitstream/43624/2/Simarouba%20brochure,%20UAS%20Bangalore,%20India.pdf.]. In first 3-4 years, during the pre-bearing period, the traditional short term annual crops such as sunflower, soybean, groundnut, pulses are chosen as intercrops depending upon type of soil and requirement of crops, shade loving crops also be grown as intercrops after gestation period. For effective pollination and good bearing, planting of high yielding and andromonoecious plants (pollen donars) at a distance of about 60.0 meters from each other among the female plants in the right geometry is advocated or some selected female plants may be grafted with high yielding andromonoecious scions. Wind and honeybees act as pollinating agents [novodboard.com database].

#### **F. Yield**

Though all parts of this plant are useful in one way or other, the seeds are important as they contain 60-70% oil [98]. Each well grown tree yields 15-30 kg nutlets equivalent to 2.5 to 5.0 kg oil and about same quantity of oil cake. [3, 75]. The annual returns in kg/ha from a moderately well managed 10 year old plantation of *Simarouba* is as follows. Oil:-1000-2000, Oil



cake: - 1000-2000, Fruit pulp: - 8000-10000, Leaf litter:-10000-15000, Shell: - 4500-9000 [3].

### G. Pathology

Generally cattle and goats do not browse on *Laxmitaru*. It has no major pests and diseases are recorded at present in its native and also under Indian conditions [1]. The almond moth *Ephestia cautella* occurs as a pest on stored decorticated seeds [29]. In conditions at Bangalore the mites (*Eutetranychus sp.*) attacking the *Simarouba* seedlings and the bark feeder (*Inderbela sp.*) are noticed on some plants. In the nursery the seedlings are affected by damping off caused by *Pythium SP.* and wilt caused by *Fusarium SP.* These diseases are generally controlled by application of commercial fungicides [98]. Armour [1], reported the sooty mold *Capnodium Species* on the 'honey' deposited by scale insects and aphids. Several species of scale insects have been found to attack the young growth of *Simarouba* and most common is *Tolumeyella SP.* Which has been controlled by using mixture of 50% malathion at the rate of 1% in water. Most common insect pest is the larva of threadworm (*Attevaergartica walshm*) feeding on the growing tips and particularly among panicles of male flowers weaving intricate webs. These insects can be readily controlled using a 5% DDT spray or Toxaphene at 20 % .He further reported that several species of scale insects, such as *Chianapsis species* and *Ceroplastes species* attack the young trees. The mites (*Brevipalpus Phoenicus Geijskes*) which has apparently induced proliferation of the apical buds, causing a "witch's broom" effect on terminal shoots. This seriously reduces fruiting and control measures using the more common miticides have not yet proved effective.

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