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Naregamia alata: An endanger medicinal plant

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Abstract
Naregamia alata (Meliaceae) is a shrub endemic to peninsular India and is found throughout Kerala. Its herbal medicine is used for the treatment of various diseases like eczema; pruritus, scabies, jaundice, anaemia, asthama, bronchitis, arthritis, biliousness, phlegm vomiting. It is alexteric, antibacterial, depurative, antipyretic, emetic, antioxidant rich, and its methanolic extract also showed hepato-protective activity. Due to excessive exploitation for medicinal use, the plant has become rare in nature. So propagation and conservation of plant is required using the tissue culture technique. This review focuses on the botanical description, phytochemistry, nutritional studies and pharmacological properties of this plant.

Keywords: *Naregamia alata*, medicinal plant, antibacterial activity, phytochemicals

1. Introduction
Indian tradition & culture is associated with the use of medicinal plants from thousands of years ago. They used to cure their diseases by using these naturally available plants in which *Naregamia alata* also plays an important role. It is an 15-20 cm tall shrub which is mainly found in the western peninsular rocks and sloppy region of India. There are different common names of this plant in different languages of India like Goanese ipecac, Goanese ipecacuanha (English), Nilanarakam, Neelanargam (Malayalam), Nilabevu (kannada), Teen parni(Hindi), Tinpani (Konkani), Neelanaaragam (Tamil), Pagapapu (Telugu), Pitmari, Pitpapra (Marathi), Amlavalli, Triparnika, Brihatpatra (sanskrit) (ref. kerala karshakan). It is called Triparni in Hindi because its leaves are trifoliate i.e, divided in three leaflets, that is wedge in shape. General habitat of *Naregamia alata* are moist deciduous forests and some areas of the plains.
Naregamia alata comes under kingdom Plantae, phylum Tracheophyta, subphylum Euphyllophytina, class supermatopsida, order Sapindales, family Maliaceae, tribe Phyllantheae, genus *Naregamia*, spp. *Alata.* It found in southern part of India like Kerala, Maharashtra etc. The name of its genus is taken from its Malabar name which is derived from two words i.e. nela means 'earth' and aregam that is 'a spp. of citrus nelanaregam' (Manilal 2003). This shrub is not of classical origin; description about the plants is not available in the Vedas but references regarding this shrub are available in Raaja Nighantu and modern books like Indian Materia Medica & Medicinal Plants, and Recent Floras Mool kandi. Form research point of view also very limited research papers are available on this topic and moreover no scientific review on *Naregamia alata* has yet been published. Due to its high medicinal value its demand is increasing day by day so it is important to conserve its genetic diversity by using multidisciplinary approaches.The frequency of distribution is very less because it is mainly found throughout Kerala, or southern states which leads to its less genetic diversification or variation. *Naregamia alata* is a well-known medicinal plant used for various purposes and coated in main index its roots are magical expectorant used in dysentery while leaves and stems are used in decoction (Raghunathan M., *et al*., 2017) [10]. The plant is also used in rheumatism and the root contain alkaloid 'Naregamin (Asha and Pushpangadan 2002) [3] having cooling and expectorant properties and useful in acute dysentery. It is also used as an expectorant.The juice from each part of the plant is pressed and rubbed with Indian walnut oil to cure itching and a drink is prepared with water pressed from the root against epilepsy with fever. The roots contain alkaloid and owing to over exploitation for medicinal uses, the plant has become rare in nature.

1.1 Botanical description
1.1.1 Plant
It is a dicot, prostrate woody shrub with shiny leaves and the height of the plant varies from 15- 45 cms. Leaves are further divided into three leaflets.

It is planted in rainy season and flowers appear in the month of May to June while the fruits are set in October month. Habitats of *Naregamia alata* are rocky and grassy slopes. It is propagated through seeds.

1.1.2 Flower
It has medium sized flowers with 5 petals which are white in colour, distinct from each other and 1.5 -1.8 cm long with capitate stigma. Flower position is auxillary and solitary, short pedicle with 5 lobed calyx each 3-4 mm long and lanceolated. Staminal tube is 1.8 -2cm long with cylindrical inflation at top. They are white in colour with 10 yellow teeth at margin. Its flowers are very attractive when it blooms.

1.1.3 Fruit
Fruit type of *Naregamia alata* is a green colored capsule. It has four locules and is ovoid in shape. Fruiting takes place in the month of October. Each cell has two seeds and they are curved and truncate on both the sides. The size of seed is approx 1mm and capsule is about 4- 5 mm in diameter.

1.1.4 Roots
Root having thick bark which contains pungency and aromatic compound. Alkaloid 'naregamin' is the main content in bark of *Naregamia alata* root.

2. Distribution
Since the frequency of genetic distribution of *Naregamia alata* is limited, it is mainly found in peninsular region of India i.e. all district of Kerala, Karnataka, Andhra and some areas of Tamil Nadu and Maharashtra. The reason behind limited distribution of this crop is lesser variation & restricted adaptability in different climatic zones.

2.1 Propagation and Cultivation
The plant can be propagated from roots, stem cuttings and seeds, micro propagation through conventional as well as in tissue culture is also reported (Vazhacharickal PJ *et al*., 2018) [17]. For conventional propagation requirement of coastal sandy soil with organic manure help enhancing growth and flowering. Watering is essential to keep the plant alive during summer season otherwise it will naturally dry out. Conventional propagation through sprouting of cuttings is slow and does not generate the required number of propagules. There is also reported adventitious shoot formation from mature leaf and leaf derived callus of this plant. Daniel M., *et al*., 1999 [12] describes a protocol for the rapid *in vitro* clonal propagation of this valuable medicinal species from stem cuttings from *Naregamia alata* plants. Shoot tips (10 mm long) from 30 days old potted plants were thoroughly washed with tap water and 10% *(v/v)* Labolene and finally surface disinfected by treating with 0.1% *(w/v)* mercuric chloride solution and rinsed with distilled water inoculated on MS medium additionally containing Salicylic acid and Gibberelic acid for shoot proliferation. For rooting, the MS medium the cultures were incubated at 25 °C under 12h photoperiod with 70 mol photons m^2s provided by fluorescent tubes. Plants were transferred to field after initial hardening on filter paper bridges in a sugar free MS basal liquid medium for 21 days and transferred to field in vermiculite in plastic cups and nourished on every alternate day with modified Hoagland's solution (Delse, P.S *et al*., 2009) [15].

3. Chemical property
It contain about 34 compounds which includes alkaloids, carbohydrates, flavonoids, carboxylic acids resins, tannin (Moh. Rishad *et al*) which were identified by mass spectral database search and comparison of linear retention indices. Vinny K Vetal., 2017 has developed a HPTLC fingerprint profiles of various secondary metabolites for methanolic extract of the root of the traditional medicinal plant, *Naregamia alata*. They used TLC scanner 3, Reprostar 3 and WIN CATS-4 softwares along with HPTLC technique and gave the information about the presence of glycosides, flavonoids, alkaloids and phenols in the methanolic root extract. They used three different mobile phases, which showed different Rf values. HPTLC finger printing of methanol extract of root in Mobile phase1 revealed 10 peaks with Rf values in the range of 0.13 to 0.92, Mobile phase 2 showed 7 peaks with Rf values in the range of 0.27 to 0.93 and Mobile phase 3 revealed 4 peaks with Rf values in the range of 0.25 to 0.78 (Table 1).

Table 1: Chemical compounds found in *Naregamia alata*

S. No.	Phytochemical	References
1	Terpenoids	Georgev M., *et al*., 2017 [4] and Supratha P.T & Saj O.P 2016 [16]
2	Glycosides	
	Coumarin	
3	Steroids	
4	flavonoids,	
5	Phenolic compounds,	
6	Alkaloids	
7	Saponins	
8	Tannin	
9	Terpenoids	

4. Nanoparticles
Leaf extract isolated from the herb *Naregamia alata* has been used for the synthesis and characterization of silver and gold nanoparticles (Francis S *et al*., 2017) [14]. For synthesis, microwave radiations are used as a source of energy, for the reduction of the metal ion precursors. The spherical silver nanoparticles have an average diameter of 18.05 ± 4.73 nm, and the poly-shaped gold nanoparticles exhibit an average size of 27.92 ± 9.19 nm. The X-ray powder diffraction patterns indicate different crystal planes, namely the (111), (200), (220) and (311) planes of nanosilver and nanogold. The actions of six major mastitis pathogens are inhibited by the antimicrobial power of the silver and gold nanoparticles. Colored pollutants from the paper, printing and textile industries such as Eosin Y and Methyl red are degraded without light irradiation using the catalytic power of the synthesized noble metal nanoparticles. Furthermore, the reduction of the organic compound 4-nitrophenol by NaBH4 in the presence of the nanocatalysts increases the laboratory value and phyto-nanoparticles have multi-functionalities due to their sustainable origin and bio-compatible nature (Francis S *et al*., 2017) [14].

5. Medicinal uses
All the parts of *Naregamia alata* are used in ayurvedic medicines. It is used in many folk medicinal treatments. *Naregamia alata* shrub is used in curing eczema; pruritus; scabies; jaundice (Asha and Pushpangadan 2002) [3]. It is also used in anaemia, asthama, bronchitis, arthritis, biliousnesss,

phlegm vomiting etc. It is alexteric, antibacterial, depurative, antipyretic, emetic, vulnerary in nature (Warrier *et al*. 1995). Apart from this it also shows the presence of hepatoprotective activity in the methanolic extract from stem of *Naregamia alata,* against carbon tetrachloride induced toxicity in rats (Sonu Jacob et l., 2012) [7] and cure snake bites also. Oil is also extracted from this plant which is good for hair. The juice of this plant is also used for liver ailments, diabetes and psoriasis (Anagha *et al*. 2013) [1].

Table 2: Phytochemical compounds isolated from the root extract of *Naregamia alata* by using different solvent (Source: Supratha P.T. and Saj O.P. 2016) [16]

Solvent	Phytochemical
Hexane extract	Steroids and Terpenoids
Ethyl acetate extract	Steroids, Alkaloids, Phenolic compounds, Amino acids and Terpenoids. Flavonoids, Proteins
Methanol extract	Alkaloids, Saponins, Tannin, Phenolic compounds, Flavonoids, Proteins, Amino acids and Carbohydrates

Table 3: Fatty acid composition of *Naregamia alata* root

S. No.	Fatty acids
1.	B-caryophyllene
2.	y-himachalene
3.	Caryophy Ilene oxide
4.	B-sesquiphellandrene
5.	p-meth-8-ene-3-ene-3-methy Jene

(**Source:** https://sg.inflibnet.ac.in/bitstream/10603/151024/15/15_references.pdf)

5.1 Antioxidant Activity

The antioxidant potential of methanolic extract of *Naregamia alata* roots were determined by using different *in vitro* assays including DPPH (2,2-diphenyl-1 picryl hydrazyl radical), free radical scavenging assay, hydroxyl radical scavenging assay, nitric oxide scavenging assay, reducing power and superoxide free radical scavenging assay kit. Ascorbic acid has been used as a standard. Supratha P.T, Oommen P. Saj 2016 [16], used different concentrations of methanolic root extract like 12.5, 25, 50, 100 and 200 ug/ml during their studies. *In vitro* antioxidant activity of the methanolic root extract showed good antioxidant power with IC 50 values of 7.5 ug/ml, 45ug/ml and 20 ug/ml as in DPPH assay, Hydroxyl radical and superoxide free radical scavenging assay respectively with ascorbic acid used as standard (Table 4).

Table 4. Antioxidant Activity of methanolic extract of *Naregamia alata* W&A, roots (Supratha P.T, Oommen P. Saj 2016) [16]

S. No	Concentration (ug/ml)	DPPH Method	Nitric oxide scavenging assay	Hydroxy radical scavenging
1	12.5	70.32	38.10	28.50
2	25	72.10	41.34	44.22
3	50	79.8	46.79	51.47
4	100	82.78	52.56	63.33
5	200	91.39	55.44	76.90

Table 5: Antioxidant Activity of methanolic extract of *Naregamia alata* W&A (Jacob S., *et al*., 2011a) show DPPH and Superoxide Scavenging method b) Reducing power assays.

(a)

S. No	Concentration (µg/ml)	DPPH Method	Super oxide scavenging assay
1	5	4.02	5.10
2	10	7.27	9.34
3	15	11.29	17.11
4	20	16.80	23.56
5	25	22.14	28.30

(b)

S. No	Concentration (µg/ml)	Reducing power assays
1	25	0.078
2	50	0.098
3	75	0.184
4	100	0.464

5.2 Antibacterial Activity

Antibacterial and antifungal activity has been found in the extracts of *Naregamia alata* plant. Extract were isolated with different solvents like petroleum ether, chloroform and methanol. In this method different gram positive and gram negative bacterias like *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebseilla pneumonia, Salmonella typhi, Serratia marcescens, Pseudomonas aureogenosa, Bacillus subtilis* were used. Sharma *et al*., 2011 [6] reported the antibacterial potential of the stem of *Naregamia alata*. The aqueous stem extract was found effective against *E.coli, P.vulgaris, P.aeruginosa, S.aureus* and *B.subtilis.* Chloroform extract of *Naregamia alata* found effective against *P. aeruginosa* and *K. pneumoniae*. Petroleum ether extract of *Naregamia alata* were highly sensitive to *P. mirabilis, B. subtilis* and *E. faecalis*. Hexane extract of *Naregamia alata* found effective against *E.coli, B.subtilis, S.typhi* and *E.faecalis.* Ethyl acetate extract of *Naregamia alata* found effective against *E.coli, S.aureus, S.marcens, P.mirabilis, K.pneumoniae,* and *E.faecalis.* Ethanolic extract of *Naregamia alata* was found effective against all the strains under study except P.*aeruginosa*. Aqueous extract was found effective against *E.coli, P. Mirabilis, B.subtilis, S.typhi, B.cereus* and *E. faecalis* of *Naregamia alata*. The antibacterial activity of the extracts increased linearly with increase in concentration of extracts (100µg/ml) as compared with control **(**Table 6). Ethanolic extracts of the plant was found most effective against all the tested bacteria. Although antibacterial activity was studied *in vitro*, the results showed that the extracts from *Naregamia alata* possess significant antibacterial activity, confirming the great potential of bioactive compounds and are useful for rationalizing the use

of this plant in primary health care. This may help the development of new antibacterial drugs from these plants. Antifungal assay of this plant is carried out against fungus like Candida *albicans* and Candida *glabrata.* In this case Sharma *et al*., 2011 [6] reported that petroleum ether extract is having highest activity than Chloroform, Methanol and control (Table 7). Sharma M *et al*., 2011 [6] also determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (mg/ml) of the alcoholic and aqueous extracts. The antibacterial potential of the *Naregamia alata* were then compared with Benzyl Penicillin and Amphicillin, two common antibiotics employed in allopathic treatment of bacterial diseases. The alcoholic extracts of plants proved to be more effective than the aqueous extracts due to broad spectrum antibiotic compounds. The good antibacterial potency of the plant indicates the presence of some active principle components in the phytoextracts, which can be purified and employed in the treatment of bacterial diseases as an alternative to the costly antibiotics (Table 8).

Table 6: Antibacterial activity of various extracts of *Naregamia alata* (Source: Jacob *et al*., 2011)

S. No.	Solvent	*Bacillus cereus*	*S.aureus*	*E. coli*	*K.pneumoniae*	*Salmonella typhi*	*Serratia marcescens*	*P.aureogenosa*	*B.subtilis*
1	Petroleum ether	8mm	12mm	9mm	8mm	12mm	12mm	7mm	10mm
2	Chloroform	10mm	10mm	10mm	10mm	20mm	14mm	13mm	14mm
3	Methanol	13mm	10mm	10mm	13mm	12mm	9mm	9mm	11mm

Table 7: Antifungal activity of various extracts of *Naregamia alata* (Source: Jacob *et al*., 2011)

S. No.	Solvent	*Candida glabrata*	*Candida albicane*
1	Pet. ether	12mm	12mm
2	Chloroform	12mm	10mm
3	Methanol	12mm	12mm

Table 8: MIC (mg/ml) of experiment plant *Naregamia alata* (Source: Sharma M., *et al*., 2011) [6]

Organism	*Leaf*		*Stem*		*Root*	
	A	E	A	E	A	E
E. Coli	1.8	0.9	0.8	2.1	1.7	0.9
P. vulgaris	2.0	0.9	0.7	1.9	1.9	0.7
P.aureogenosa	2.3	1.2	0.7	2.3	1.5	0.7
S.aureus	1.7	0.2	0.8	2.9	2.0	0.9
B.subtilis	1.5	1.8	0.7	3.0	1.8	0.8

A= aqeous; E= Ethanol

5.3 Hepato-protective Activity

Ashai V.V and Pushpangadan P. 2002 [3], share the ethnomedical information of *Naregamia alata* drugs against liver diseases. Stephen Ambrose S *et al*., 2012 [5] did the clinical trials of these drugs in experimental animal models and provide the efficacy and safety of these plant drugs against hepatoprotective activity

5.4 *In vitro* Anti-inflammatory Activity

Studies on the anti-inflammatory activity of the methanolic extract of *Naregamia alata* were carried out using different methods by George M *et al*., 2017 [4]. They found that the plant extracts were effective against inflammation due to the% inhibition properties which were compared using different methods like inhibition of albumin denaturation, antiproteinase assay, HRBC membrane stabilization and heat induced haemolysis by using Diclofenac sodium, Aspirin, Indomethacin and Aspirin as standard respectively. The highest% inhibition was shown at the concentration range of 500 µg/ml. The highest inhibition was shown by the extracts in the albumin denaturation and antiproteinase assay methods and its anti inflammatory activity is found to be significant (Table 9).

Table 9: The *in vitro* anti inflammatory activity (% inhibition) by different methods (Source: George M *et al*., 2017) [4]

S. No	Naregamia Methanolic extract (ug/ml)	Inhibition of Albumin Denaturation (Diclofenac Sodium)	Antiproteinase Assay (Aspirin)	HRBC Membrane Stabilization Method (Indomethacin)	Heat Induced Haemolysis (Aspirin)
1	100	33	21	21	30
2	200	50	30	27	48
3	300	61	36	36	54
4	400	69	43	42	61
5	500	78	51	53	75

5.5 Flavonoids present in *Naregamia alata*

Undarathi J. 2017 [9] identified the bioactive flavonoids from *Naregamia alata*, which were further confirmed by *FT-IR and UV spectroscopy.* These studies shall open new pharmacological avenues for this magnificent plant. A proper phytochemical and pharmacological study is important for scientific validation and such studies are also crucial for clinical experimentation and in the development of novel drugs. Undarathi J. 2017 [9] experimental data reveals that the plant contains flavonoids as the major active principle and show the hepatoprotective activity.

Table 10: Flavonoid Classes of *Naregamia alata (*Undarathi J. 2017) [9]

S. No	*Flavonoids*	Principal maxima (nm)
1	Anthocyanins	475-560
2	Aurones	390-430
3	Chalcones	365-390
4	Flavonols	350-390
5	Flavonols	250-270
6	Flavones and biflavonyls	330-350
7	Flavanones and flavanonols	275-290
8	Flavones and biflavonyls	250-270
9	Isoflavones	255-265

6. Conclusion

Naregamia is a treasury assets of South India. *Naregamia alata* is a very slow growing shrub with small thorny stem. *Naregamia alata* has immense therapeutic values, therefore, it is used excessively and unscientifically by the local rural people for medicine that has led to extensive over exploitation and depletion of the species from nature making the plant vulnerable. The other but important reason behind the endangerment is that *Naregamia* is very less diversified medicinal plant and it is having geographically restricted habitat. Due to this less variation found in *Naregamia alata* in comparison to broadly distributed spp. Further research should be need to done to conserve this valuable, precious medicinal plant.

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