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## A Review and Acute Oral Toxicity Study of *Asystasia variabilis* Trim- A Medicinal Plant used by Folklore Practitioners

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### ABSTRACT

**Introduction:** Ayurveda believes every plant is medicinal. *Asystasia variabilis* Trim. belonging to the family *Acanthaceae*, called as *Maithaalakaddi* by folklore practitioners of Udupi. It is a semi scandent herb, generously growing in rainy season throughout coastal region, which is used effectively by the Folklore practitioners to treat different *pitta vikaras*. Considering its therapeutic potentialities the drug is reviewed and to establish its safety profile the Acute Oral Toxicity study is carried out. **Methodology:** The literary review was carried out by collecting the relevant material from *Vedas*, *Samhitas* and *Nighantus*. Information about the drug in recent day is reviewed from relevant textbooks, journals and relevant websites. The whole matured plant of *A. variabilis* was identified, collected to prepare churna (powder) and AOT study was carried out as per OECD-425 guidelines. **Results:** Although, various therapeutic applications are mentioned in detail in various ethno-medico-botanical surveys which are published, the drug is not considered as a source for any of the drug mentioned in *Samhita* or *Nighantus*. The acute oral toxicity study shows (LD<sub>50</sub>) is much more than 2000mg/kg. **Conclusion:** The plant is considered as new to Ayurveda and it is proved it to be a safe drug.

### KEYWORDS

*Acute Oral Toxicity study, AsystasiavariabilisTrim., Folklore practitioners, Maithaalakaddi*



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## INTRODUCTION

Since ancient times, man has looked towards nature for drugs to cure diseases. In the beginning the use of medicinal plants were based on instinct. At that time treating diseases was based on experience as there was not enough information regarding the reason for illness or which plant to use to cure it. With time the reason for using specific medical plants were discovered for treating certain diseases. During recent years traditional medicinal system has become a topic of importance at global level. Estimates show that a large number of populations from many developing countries are relying of traditional practitioners for their health care needs. Even though modern medicines are available, traditional medicines have gained the popularity. The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996<sup>1</sup>.

India is a country rich in diverse heritage and cultural traditions. Such rich traditions are associated with use of herbal plants for medicinal use. Even now the traditions of using medicinal plants are still adopted by the local communities who are still in living in dense forest. Medicinal plants are the “backbone” of traditional medicine, which

means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis<sup>2</sup>.

*Acharya Vagbhata* mentioned that plants available in vicinity are much beneficial in the management of disease<sup>3</sup>. *Asystasia variabilis* Trim. also known as *Maithaalakaddi*, is one such plant, belonging to the family *Acanthaceae*, generously growing throughout Udupi district. It is effectively used by the Folklore practitioners for the management of *pittaja vikaras*, rheumatism and worm infestation. Traditionally the whole plant is squeezed in the water, which is then used to grind the rice to prepare a traditional dish called *Manni*, which is specially consumed during *Sharad rutu*, as it is considered as *pitta prakopa kala*<sup>4</sup>.

As the plant is used commonly available and used extensively by the folklore practitioners the study about the identity and the pharmacological actions are necessary. Thus to give a scientific evidence to the plants safety, acute oral toxicity study was conducted.

## MATERIALS AND METHODS

Ethical Committee Approval Number -  
CPCSEA/IAEC/SDM-DG-07 Dated:  
11/09/2017



**Literary review: Source of data:** The information about the drug was obtained from various Ayurvedic samhitas like *Bruhatrayis* and *Laghutrayis*. The reference of the drug was also checked from Nighantus. For its exact identification and botanical description various floras were referred. For collecting the information regarding its medicinal properties relevant textbooks, various publications, journals and websites were referred.

**Acute oral toxicity study<sup>5</sup>: Conditions- Animal species-** Rats, **Strain:** Wistar albino, **Source:** Animal house attached to SDM Research centre, SDM Ayurveda College, Udupi, **Selection:** A total of 5 healthy rats of either sex of body weight 250-350 g were selected according to AOT software, **Acclimatization Period:** All the selected rats were kept under acclimatization for 7 days before dosing, **Numbering and identification:** The rats were marked with saturated Picric acid solution in water for identification, the group number, animal number and sex of the animal were identified with the help of cage cards, as presented in the Table 1.

**Husbandry conditions: Housing-** Rats were housed in each cage of polypropylene with stainless steel top grill. The paddy husk was used as bedding material and was changed every morning. **Environment-** The animals were exposed to 12 hrs light

and 12 hrs dark cycle with the relative humidity 50-70 % and the ambient temperature  $23 \pm 0.30^{\circ}\text{C}$ . **Diet-** SaiDurga Feeds Bangalore provided food to rats throughout the study period except on previous night of dosing i.e. (overnight) fasting before dosing. The drinking water was given *adlibitum* polypropylene bottles with stainless steel sipper tube.

**Preparation of test formulation for administration: Test drug-** *Asystasia variabilis* Trim. **Vehicle-** Water, **Dose preparation-** The test formulation was, water extract of *A. variabilis* whole plant was prepared. All rats were given dose (0.5ml /100 g body weight), **Schedule-** Single dose per rat, **Administration-** The test drug was administered through oral route at different dose levels to respective animal through oral feeding needle on to disposable syringe, **Dose fixation-** According to AOT software, **Route-** Oral, **Dose-** 175 mg/kg, 550 mg/kg, 2000 mg/kg test substance, **Dose volume-** 0.5ml /100 g - animal.

## RESULTS

*A. variabilis* has not been considered as a drug source in any of the Ayurveda literatures like *Vedas*, *Samhitas*, *Nighantus*, etc till date The vernacular names of the plant are mentioned in Table 2.



**Botanical information:** *Asystasia variabilis* Trim. syn. *Asystasia chelonoides*(Nees) var. *quadrangularis* C.B.Clarke<sup>6</sup> belongs to Acanthaceae family. The taxonomical position is mentioned in Table 3.

**Table 2** Vernacular names of *Asystasia variabilis* Trim.

Language	Name(s)
Kannada	<i>MaithalaaKaddi</i>
Tulu	<i>MaithalaaKaddi</i>
English	Salt mucilage vine
Sinhalese	<i>GadaPuruk</i>
Malayalam	<i>Murikootipacha</i>

**Table 3** Taxonomical position of *A. variabilis* Trim.

Kingdom	Plantae
Division	Angiosperms
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Scrophulariales
Family	<i>Acanthaceae</i>
Genus	<i>Asystasia</i>
Species	<i>A.variabilis</i>
Species authority	Trimen, Henry

The morphological description of this drug, it's different species and distribution are described in Flora of South Canara<sup>7</sup>, Flora of Presidency Madras authored by Gamble.J.S, Flora of British India Vol4 authored by Sir J.D. Hooker and C.B., K.C.S.L, Flora of Ceylon Vol 12 authored by Dassanayake M.D. and Clayton.

In Flora of British India, Clarke proposed a new variety of *Asystasia* viz., *Asystasiachelonoides* var. *quadrangularis*. Subsequently, Gamble 1924; Mohanan in 1984; Vajravelu in 1990; Mohanan & Henry in 1994 have also cited this plant in Kerala part of Western Ghats and Matthew

in 1991 from Tamil Nadu. Later, this taxon was reduced to synonymy of *Asystasia chelonoides* Nees without proper investigation of the type material. After the most recent treatment of *Asystasia* genus in India in 2013, Sasidharan continued the usage of variety *quadrangularis* as belonging to *Asystasia chelonoides* Nees, followed by the Global Biodiversity Information Facility in 2016.

In the year 1895, Trimen made a combination *Asystasia variabilis* (Nees) Trim. in Handbook of Flora of Ceylon to treat a Sri Lankan species, based on *Ruellia variabilis* and *Asystasia gangetica* var. *variabilis*. The name is an accepted name but confined to Sri Lanka. Critical examination on large number of herbarium sheets and pertinent literature revealed that the specimen from India belonged to *Asystasia variabilis*. In fact, the south Indian *quadrangularis* variety and Sri Lankan *A. Variabilis* are currently assigned to single taxon, *Asystasia variabilis*<sup>6</sup>.

**Family characters- Acanthaceae<sup>7,8</sup>**  
*Acanthaceae* word meaning is a natural order of plants, chiefly tropical, consisting of soft wooded herbs, undershrubs, and shrubs: many of them very beautiful. Herbs or shrubs; stems erect, ascending, twining or prostrate, sometimes armed with spines. Leaves simple, opposite, estipulate, mostly with cystoliths. Flowers irregular, bisexual,



in spikes, racemes or cymes, rarely solitary; bracts large or small, sometimes 0; bracteoles usually 2. Calyx 4-5-partite, sometimes small and multifid present in fruit. Corolla gamopetalous, 2- lipped or sub equally 5- lobed, sometimes upper lip reduced or absent: lobes imbricate or contorted in bud. Stamens 4, didymous, sometimes 2, epipetalous, included or exerted; anthers 2 or 1-celled, if 2- celled cells parallel or inserted at unequal levels, spurred or not. Ovary superior, 2 celled; ovules 1 or more in each cell, axile; style elongate; stigma notched or bifid. Fruit a loculicidal capsule. Seeds 2-many, hard, generally attached to up curved supports (retinacula), ovoid or compressed, usually non endospermous.

**Genus characters– *Asystasia*<sup>7, 8, 9</sup>**

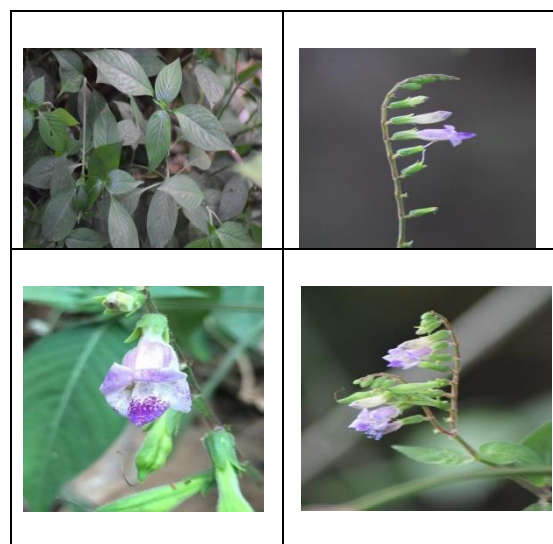
*Asystasia* means inconsistency and relates to the fact that corolla is more or less regular which is unusual in the family *Acanthaceae*

Erect or straggling herbs or under shrubs. Leaves usually entire. Flowers in lax or dense, usually 1-sided, simple or compound spikes or racemes, bracts and bracteoles small, similar. Calyx 5-partite. Corolla – tube cylindric below, inflated above, ventricose or funnel- shaped: lobes 5, subequal, imbricate in bud. Stamens 4, included: anthers oblong, cells parallel. Ovary 2-celled: ovules 2 in each cell.

Capsule elliptic, stipitate. Seeds 4, compressed, rugose or tuberculate: retinacula hooked, strong.

**Key characters of genera** –Flowers in lax, 1-sided spikes or racemes

**Species characters-<sup>6,7</sup>- Key to the species for identification-**Stems is acutely quadrangular; corolla less than 2cm long. Inflorescence- Racemes terminal, lax, 7-13 cm long, compact, 4-6 cm long; pedicel 1.5 mm, bracteoles similar linear, 0.5 cm long, green, acute, pubescent, persistent. (Figure 1)



**Fig 1** The Habit, Leaf, Flower, Fruit of *Asystasia variabilis* Trim.

**Habitat and Distribution-** It is endemic to India & Srilanka<sup>10</sup>.

**Indian distribution of *A.variabilis*** –In the coastal region of Karnataka like Udupi, South kanara, Mangalore and Kerala like Idukki, Thiruvananthapuram, Palakkad, Kozhikode<sup>10</sup>.





**Morphology**<sup>6,7</sup>- A semi- scandent herb;  
**Stems** - acutely quadrangular. Stem erect at first up to 1 m high, then semi scandent, sharply grooved on opposite sides, slightly tumid above nodes.**Leaves**- variable, up to 12 x 5 cm, elliptic, ovate or narrowly lanceolate, acuminate at apex, base narrowed, glabrous: tip acuminate, thinly hairy above, glabrous below, lateral veins 7- 8 pairs; petiole up to 2 cm long. **Flowers**- Calyx –lobes linear- lanceolate. Corolla tube funnel- shaped up to 1.8 cm long bent at base pubescent, pale pinkish–violet, dotted red. Calyx divided to the base or nearly so, lobes 5, linear-lanceolate, 6-8 mm long, acute, densely pubescent on both surface, lower midlobe border with two raised ridges. Stamens 4, didynamous, included filaments 5-7 mm long, with few small hairs, connate at the base in pairs; anthers oblong, 2-3 mm long. Ovary 2cm long, pubescent, 2 celled, ovules 2 in each cell; style linear, 1.5-1.8 cm long, basal portion hairy; stigma 2-fid or subcapitate. **Inflorescence** -Racemes terminal, lax, 7-13 cm long, compact, 4-6 cm long; pedicel 1.5 mm, bracteoles similar linear, 0.5 cm long, green, acute, pubescent, persistent. **Capsule**- up to 2.5 cm long, narrowly clavate, densely pilulose. Opening widely in a reflexed curve, **Seeds**-ovate-deltoid to orbicular, shallowly tuberculate, seeds 4, 3-

4 mm. across, Common in secondary forests.

**Phenology**- Flowering- more or less throughout the year, Fruiting – January to March<sup>16</sup>, Flowering class – Dicot habit shrub<sup>10</sup>.

**Chemical composition:** Phytochemical analysis of entire plant of *Asystasiavariabilis* showed Alkaloids, Carbohydrates, Steroids, Tannins, and Phenol<sup>11</sup>.

**Folklore / Indigenous medicinal uses**<sup>12-17</sup>: Folklore uses collected in Udupi- Traditionally the whole plant is squeezed in the water and used to grind the rice and used internally in the form of dishes. Therapeutically the same water added with jaggery used in *pitta vikaras* by the folklore practitioners in Udupi District. Entire plant folded and kept in Pickle jar helps to remove the *Lavana doshas* and a good preservative agent too. Useful in Abscesses, Cancers, Ulcers and Wounds. Stems crushed to extract the juice taken early in the morning for intestinal worms in humans. The roots are taken made into paste form boiled with coconut used in eczema in humans. Leaves of *Aloe vera*, *Antides mamenasu* and *A.variabilis* ground with egg yolk for bone fracture in Veterinary Medicines. The whole plant paste topical application in conditions like skin rashes, body irritation, swellings.



Ethno-medicines of Kanis in Kalakkad – Mundanthurai Tiger Reserve, Tamil Nadu- where noted that 1 gm of paste of equal quantities of leaves and flowers of *Asystasia chelonoides* is consumed with honey twice a day for 5 days for giddiness and dehydration and leucoderma. During the intake of *A. variabilis* the consumption of fish, egg, dried tamarind is prohibited.

**Conservation concern details:** Least concern

**Cultivation and Propagation:** As per the folk informers this plant grows in semi-shade as well as in sunny spots where it receives adequate moisture. In any soil garden it can be planted. Propagation can be done from stem cuttings, roots also from seeds. It grows commonly in farms of Areca and Coconut as a weed.

**Acute oral toxicity study<sup>18</sup>:** Examination of physical and behavioural changes-The animal was observed continuously for 4 hours after the dosing. The careful cage side observation was done without disturbing the animal attention and at the end of the every hour the animal was individually exposed to open area for recording the behavioural changes like increased or decreased motor activity, convulsions, Straub's reaction, muscle spasm, catatonia, spasticity, ophisthotonus, hyperesthesia, muscle relaxation, anaesthesia, arching and rolling, lacrimation, salivation, diarrhoea,

writhing, mode of respiration, changes in skin colour, CNS depression-hypo activity, passivity, relaxation, ataxia, narcosis etc. Mortality- all the animals were observed at 1, 2, 3, 4, 24 hours after dosing and there after daily once for mortality during the entire period of the study i.e. 14 days. Physical and behavioural examination- There were no physical and behavioural changes except mild increase in motor activity in 2 rats in the group 2000mg/kg in all treated animals on day one at 1, 2, 3, 4 hours intervals after dosing and there after once daily for 14 consecutive days. Mortality- All the rats belonging to the treated group survived throughout the 14 days observation period after dosing. From the study it was inferred that the is much more than 2000mg/kg.

**Pharmacological activities:** In vitro antioxidant studies with ethanolic extract of *Asystasia chelonoides* leaves showed significant free radical scavenging activity in DPPH (2,2 diphenyl-1-picrylhydrazyl, dark coloured crystalline powder composed of free radical molecules) and FRAP assay (Ferric Reducing Ability of Plasma) and IC (Inhibitory Concentration) values were calculated to be 300µg/ml 50 and 250µg/ml respectively<sup>19</sup>.

## DISCUSSION





*Asystasia variabilis* Trim. known as *Maithaalakaddi* by local people of Udupi is effectively used by the Folklore practitioners for the management of *pittajavikaras*, rheumatism and worm infestation. Traditionally the whole plant is squeezed in the water, which is then used to grind the rice to prepare a traditional dish called *Manni*, which is specially consumed during *Sharadrutu*, as it is considered as *pitta prakopakala*.

It is semi-scandent herb, stems are acutely quadrangular. Flowers are pale pinkish-violet, dotted red small flower, leaves variable, elliptic, ovate or narrowly lanceolate, acuminate at apex. Another species of the same genus that is *Asystasia gangetica* called *AithaalaKaddi* in Kannada/ Tulu, very commonly, abundantly available in Udupi Dist. This species is much popularly known by people and it is also used by folk practitioners. Botanically *A. gangetica* is differentiated with features such as large flowers which are varied in colour from pale white, yellow to purple and leaf is comparatively smaller than the flowers but in *A. variabilis* the flowers are smaller than the leaf and presence of purple blotches on the flowers is the key differentiating factor.

By the Acute oral toxicity study it is inferred that there were no physical and behavioural changes except mild increase

in motor activity in 2 rats in the group 2000mg/kg in all treated animals on day one at 1,2,3,4 hours intervals after dosing and there after once daily for 14 consecutive days. The drug administered is not likely to produce any sort of toxicity on acute administered even on comparatively higher doses. So with the help of this study it is concluded that the LD<sub>50</sub> is much more than 2000mg/kg drug is safe for human use.

## CONCLUSION

A systematic documentation is required and also measures should be taken to protect these priceless medicinal resources as they are at the edge of extinction. *Acharyas* have suggested making use of drugs which are found in the vicinity after careful examination and they should be included in *Ayurvedic* materia medica. So studying a locally available drug is an added benefit to the existing knowledge to provide a scientific base.

*Maithaalakaddi* is the local name of the drug and used in *pittajavikaras* by Folklore practitioners in and around Udupi district, Karnataka, India. It was identified as *A. variabilis* and it is a member of *Acanthaceae* family. *A. variabilis* has not been considered as a drug source in any of the literatures like *Vedas*, *Samhitas*, *Nighantus*, etc till now. So it is considered



as extra pharmacopoeial drug– *anukta dravya*.

The drug administered is not likely to produce any sort of toxicity on acute administration so drug is safe for human use. Further pre-clinical and clinical studies are to be carried out with this safety data to prove the therapeutic potentialities of the plant.



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