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# Authentication and Proximate Analysis of *Madhuka indica* Gmel. - A Wild Edible Flowers Used for Traditional Fermentation of Ayurvedic Biomedicines

Juliet Elizabeth Castelino<sup>1</sup>, Mallya Suma V<sup>2</sup>, KN Sunilkumar<sup>3</sup>

<sup>1</sup>PG Scholar Department of Dravyaguna, SDM College of Ayurveda, Kuthpady, Udupi, Karnataka. <sup>2</sup>Associate professor, PG studies in Department of Dravyaguna, SDM College of Ayurveda, Kuthpady, Udupi, Karnataka. <sup>3</sup>Senior research officer, SDM Centre for research in Ayurveda and Allied sciences, Udupi, Karnataka.

\*Correspondence: Email: sumamallya@gmail.com, Mobile: +919844740027

## ABSTRACT

**Introduction:** *Madhuka indica* Gmel. (Sapotaceae) is a medium sized deciduous tree, the flowers of which are commonly known as *Madhukapushpa*. These wild flowers are said to possess rich source of nutrition apart from possessing many therapeutic properties. Shade dried flowers added as fermentation initiators in *Asava-arishtha* (fermented alcoholic preparations). Hence pharmacognostic study on this flower has been designed along with its proximate analysis. **Methods:** Flowers collected from the authenticated trees were used for the study, after proper cleaning from extraneous matter. Macro-microscopy, physicochemical standards and nutraceutical composition studied using standard methodology. **Results:** Cream coloured pleasant smelling corolla; thick epidermal cuticle which packs within itself sugary, pulpy and parenchymal cells- are the characteristic Macro-microscopic features of flower respectively. Physico-chemical Standards obtained in this study will help authentication of the sample. Nutritional assessment of flowers disclosed these to be rich in both reducing sugar (31.25% w/w) and non-reducing sugar (20.625% w/w). **Conclusion:** Flowers of *Madhuka indica* Gmel. having rich pharmaceutical and nutritional benefit have investigated scientifically to record their proximate analytical value along with macro-microscopic standards. Results drawn out of this study will prove as standard parameters to authenticate the sample.

## KEYWORDS

*Madhuka indica* Gmel, pharmacognostic, proximate analysis, standardisation.

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*Madhuka indica* Gmel. from Sapotaceae is a medium sized deciduous tree found in mixed deciduous forests throughout India<sup>[1]</sup>. Flowers of this tree known as *Madhuka pushpa* cream coloured fragrant corollas collected out of this tree are of edible rich with multiple nutrient factors<sup>[2]</sup>, apart from this used as fermentation agents in alcoholic pharmaceutical preparation (*Asava-arishtha*) of Indian system of medicine<sup>[3]</sup>. The tree showers during March to April and these succulent fragrant corollas will be collected and spread in open air for drying<sup>[4]</sup>. During this flowers shrink in size, turn reddish brown and odour increases due to drying<sup>4</sup>. Flowers are eaten raw or cooked as literature reveals as a rich source of nutrition<sup>[5]</sup>. These said to contain multiple nutrient factors like sugar, vitamins, phosphorous, calcium, iron, magnesium and copper<sup>[6]</sup>. The sugar compound present in this flower is identified as sucrose, maltose, glucose, fructose, arabinose and rhamnose<sup>[7]</sup>. Flowers are used in the preparation of distilled liquors since ages<sup>[8]</sup>.

Beside this flowers found to be useful certain pathological conditions like *Rakthapitta*, *Trisna*, *Daha*, *Shrama*, *Shwaasa*, *Kshata* and *Kshaya*<sup>[9]</sup>. These are recommended in pacification of Vata and pitta dosha<sup>[10]</sup>. Ethnomedical record recommends decoction of flower in cough, chronic bronchitis and wasting diseases. Flowers advised to consume with milk in impotence due to general debility<sup>[11]</sup>.

Such a potent flower of natural origin is less studied or known to whole science of natural product. Hence a scientific study on this flower designed to document its pharmacognostic characters along with its nutritional factors.

## MATERIALS AND METHODS

### Collection and identification of plant samples

Flowers of *Madhuka* (*Madhuka indica* Gmel.) were collected in flowering season from Udupi district, cleaned properly from extraneous matter, authenticated using floras and botanists opinion, and sample deposited at SDM Centre for Research in Ayurveda and Allied sciences. (Voucher No. 15070101). Sample preserved in fixative solution. The fixative used was FAA (Formalin- 5ml+ acetic acid 5ml + 70% ethyl alcohol- 90 ml). The materials were left in FAA for more than 48 hours.

### Macroscopy

The external features of the test samples were documented using Canon IXUS digital camera. Organoleptic features of flowers like colour, taste, appearance, smell were recorded according to standard guidelines<sup>[12]</sup>.

### Microscopy

The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars<sup>[13]</sup>.

### Proximate analysis

Physico-chemical standards of flower like total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive were detected according to standard methodology<sup>[14]</sup>. Estimation of total fat, crude fibre, total protein, reducing and non-reducing sugar, total sugar carried out and values recorded<sup>[15]</sup>.

## RESULTS AND DISCUSSION

### Macroscopy

Flowers in dense fascicle near end of branch, consists of 10-12 lobed, gamopetalous fleshy cup shaped, tubular, cream coloured corolla, 1.5cm long, scented caducous, free and hairy above, flat and hollow at the base enclosing 20-25 epipetalous stamens arranged in rows. Pedicels 2.5-4cm long, densely ferruginous pubescent. Sepals ovate- lanceolate, ferruginous pubescent. Corolla usually 7-9 lobed, erect, ovate-lanceolate creamy white. Stamens 25-26, in rows. Ovary red tomentose. Style long, exerted. Anther sub sessile, basifixed, lanceolate, with pointed tip and hairy at the back with prominent dark brown connective strand (Figure 1). Taste sweet, odour characteristic.

**Figure 1. Macroscopy of *Madhuka indica* Gmel. Flowers**



### Microscopy

**Stalk:** TS is circular in outline, shows an epidermis formed by thick walled slightly papillose cells with cuticle; 2 to 5 layers of collenchymas follows the epidermis; cortex is formed by thick walled parenchyma in about 10 layers, many cells filled with some contents; inner to cortex continuous ring of phloem, separated from xylem by cambium, with usual elements are seen; the centre of TS is occupied by pith formed by parenchyma, many with contents (Figure 2).

**Calyx:** TS shows upper and lower epidermis with plenty of bent unicellular covering trichome. Inner to epidermis there are 2 to 3 layers of collenchymas having parenchymatous cortex inner to it. Lamina portion is made up of parenchyma cells similar to those found in cortex of stalk, many of the cells contain some contents and there are some cells with starch grains, yellow colored fluid and prismatic crystals (Figure 3).

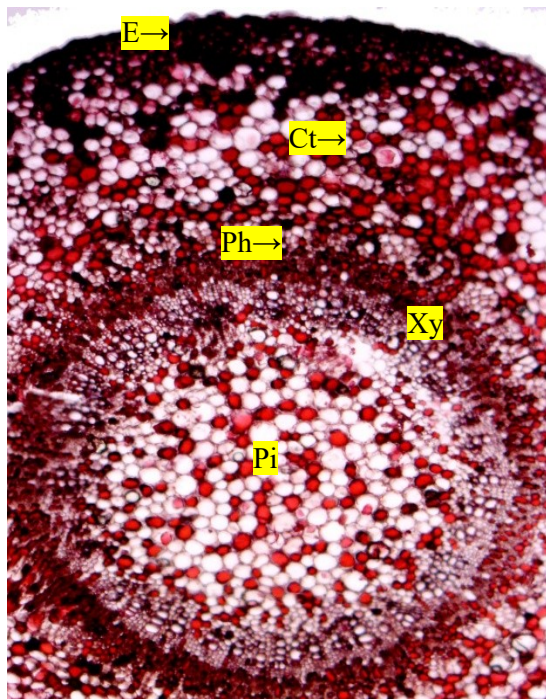
**Corolla:** TS passing through midrib shows thick walled outer and inner epidermis with cuticle, the ground tissue is made up of compactly arranged parenchyma cells full of sugary pulp and few idioblasts which are larger than usual cells and shows some contents; many tiny vascular bundles distributed throughout the section (Figure 4).

### Proximate analysis

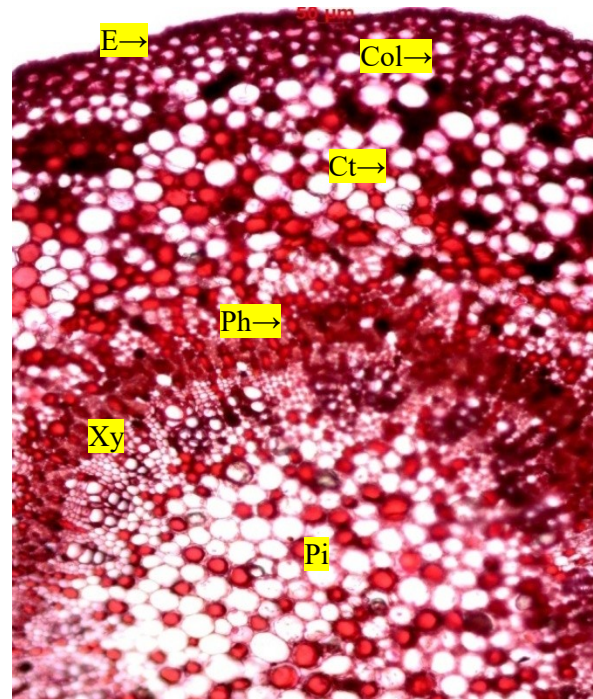
Physico-chemical and proximate analysis revealed nutraceutical values of shade dried flowers (Table 1).



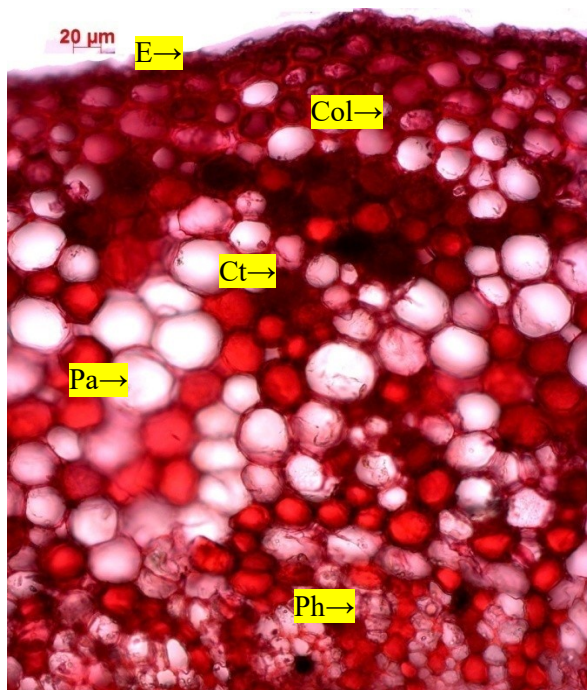
Figure 2. Microscopy of stalk of *Madhuka indica* Gmel. flowers



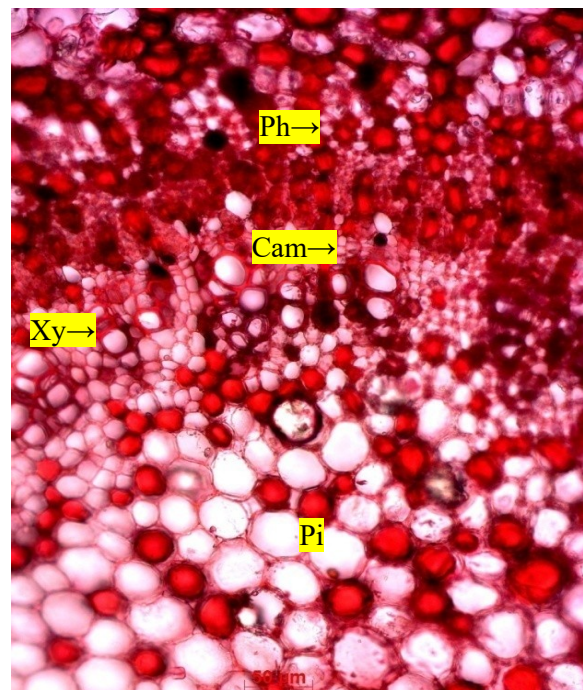
TS of stalk



A portion enlarged



Cortex and Phloem



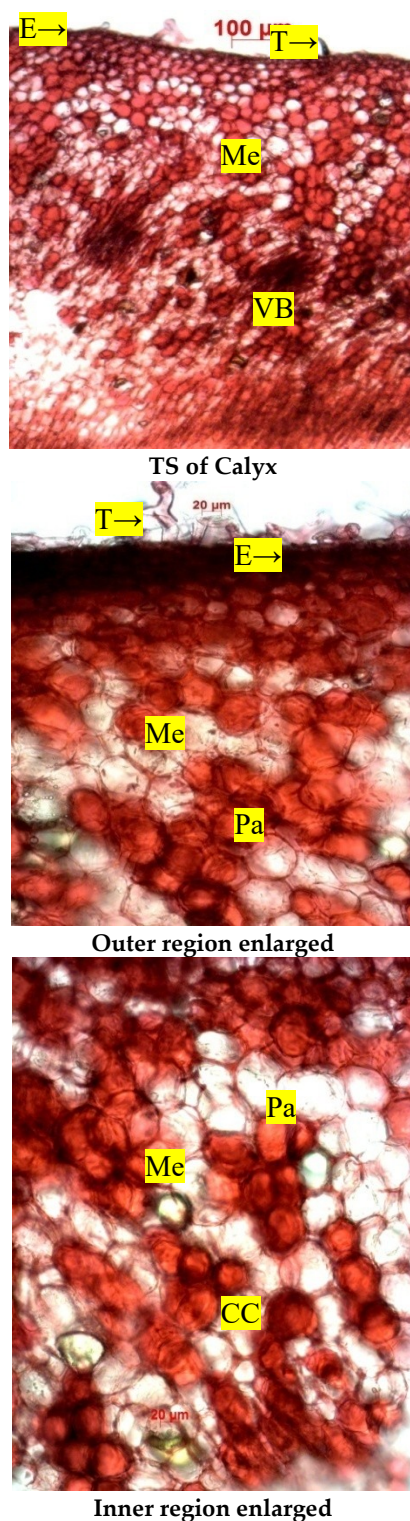
Phloem, Xylem and Pith

Cam – cambium; Col – collenchyma; Ct – cortex; E – epidermis; Pa – parenchyma; Ph – phloem; Pi – pith; Xy – xylem.

India is a great heritage of natural product and different traditional medicinal systems with innovative practicing principles. According to some records Indian material includes about 2000 drugs of natural origin. Among these 400 are of mineral and animal origin while rest are of vegetable origin<sup>20</sup>. Overuse of synthetic drugs, their adverse effects motivated human population to turn towards nature. *Madhuka indica* a wild tree with fragrant flowers is less known drug. Flowers are commonly used fermentative initiators in the preparations of *Asava- arishta.*, apart from having rich nutritional value are also used in certain therapeutic conditions. Such a valuable medicinal flower needs a quality control parameter published scientifically along with its nutritional value.



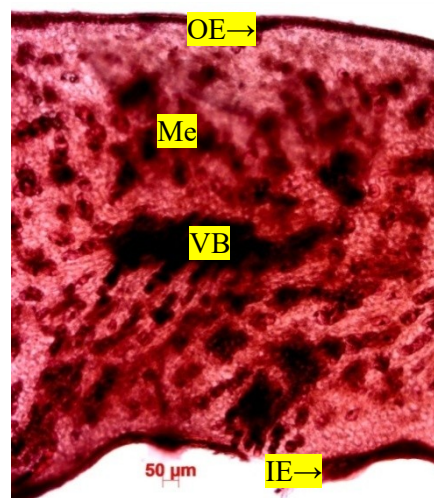
**Figure 3. Microscopy of calyx of *Madhuka indica* Gmel. flowers**



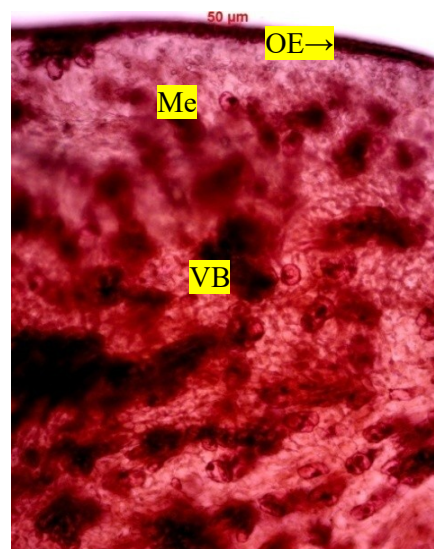
CC – content cell; E – epidermis; Me – mesophyll; Pa – parenchyma; T – trichome; VB – vascular bundle.

Authentication of raw material an important step in quality control, involves taxonomic identification, macro- microscopic recordings of the sample collected<sup>21</sup>. Macroscopy help in quick identification of plant material whereas microscopy of the drug reveal about its histological arrangement<sup>22</sup>. Cream colored 10-12 lobed gamopetalous, fleshy cup shaped tubular scented flowers are macroscopic features of *Madhuka indica* flowers. Pedicels were long (2.5 to 4cm) ferruginous and pubescent in appearance. Twenty five to twenty six Stamens arranged in rows with red tomentose ovary and long exerted style were the characteristic feature inessential whorls of these flower. Sweet taste with characteristic pleasant odour and sticky feel are main organoleptic features of the flower.

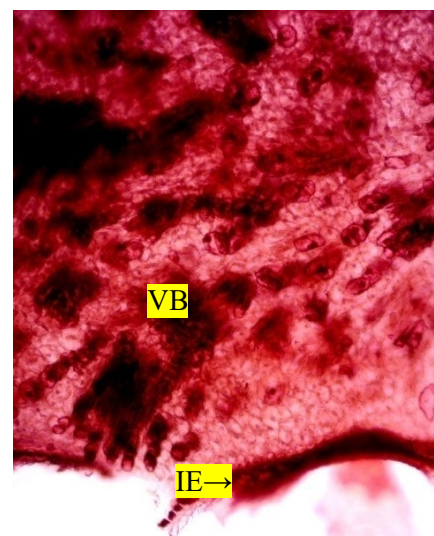
**Figure 4. Microscopy of corolla of *Madhuka indica* Gmel. flowers**



**T.S of Corolla**



**Outer region enlarged**



**Inner region enlarged**

**IE** – inner epidermis; **Me** – mesophyll; **OE** – outer epidermis; **VB** – vascular bundle

**Table 1. Proximate analysis *Madhuka indica* Gmel. Flower**

Parameter	Results n=3 %w/w
Total Ash	2.644
Acid Insoluble Ash	0.097
Water soluble Ash	0.406
Alcohol soluble extractive	23.709
Water soluble extractive	25.662
Fat	0.392
Fibre	0.139
Total protein	1.538
Reducing sugar	31.25
Non Reducing sugar	20.625

Microscopic study reveals thick walled papillose cuticle followed by collenchymatous cells of 2-5 layers are the characteristic observation of TS of the stalk of the flower. Ten layered parenchymatous cells filled with contents are the features of cortex which enclose ring of phloem separated by xylem. Centre of the stalk is occupied by pith, formed by parenchymatous cells. Unicellular trichomes are the features of TS of calyx. On its upper and lower epidermis, 2 to 3 layers of collenchymatous followed by parenchymatous cortex, filled with starch grain, yellow coloured fluid and prismatic crystals are the features of middle layer of calyx of flower.

TS of corolla characteristically shows thick cuticle of upper and lower epidermal cells, packed compactly arranged parenchymatous cells with full of sugary pulp and idioblasts with scattered tiny vascular bundles.

Physicochemical parameters of a drug are indicative off their chemical nature, contamination with physical impurities and their solubility in different extractive media<sup>23</sup>. The total ash represents the carbonaceous matter of a drug<sup>24</sup>. Here the value obtained of *Madhuka indica* was 2.644% w/w. Acid insoluble ash indicates the percentage of trace elements along with contamination of other siliceous earthy matter<sup>14</sup>. The test drug has showed 0.097% w/w represents its less contamination simultaneously showing its purity. Water soluble ash obtained from the test drug was 0.406% w/w. Alcohol and water soluble extracts represents their solubility in respective media along with the chemical nature of a drug, as well as maximum extractive principles that can be obtained in these media<sup>25</sup>. Here the values obtained are 23.709 %w/w and 25.662% w/w in alcohol and water respectively shows their solubility in these media.

Proximate analysis is a system of analysis of nutrients also termed as Conventional analysis in which gross components (protein, fat, carbohydrate, ash) of the material are determined<sup>26</sup>. *Madhuka* flowers are one among such edible flowers and are consumed as a source of nutrition. Proximate analytical report of test drug supported the view as a source of protein (1.538%), reducing sugar (31.25%) and non-reducing sugar(20.625%).

## CONCLUSION

*Madhuka indica* Gmel, a wild tree with edible fragrant flowers, used commonly in Indian system of Medicine in the preparation of Asava-arishta. Rich commercial, nutritional and medicinal values of these flowers tested scientifically and documented systematically. Nutritional value along with its pharmacognostic parameters published in this paper will prove as authenticated quality measures of test drug.

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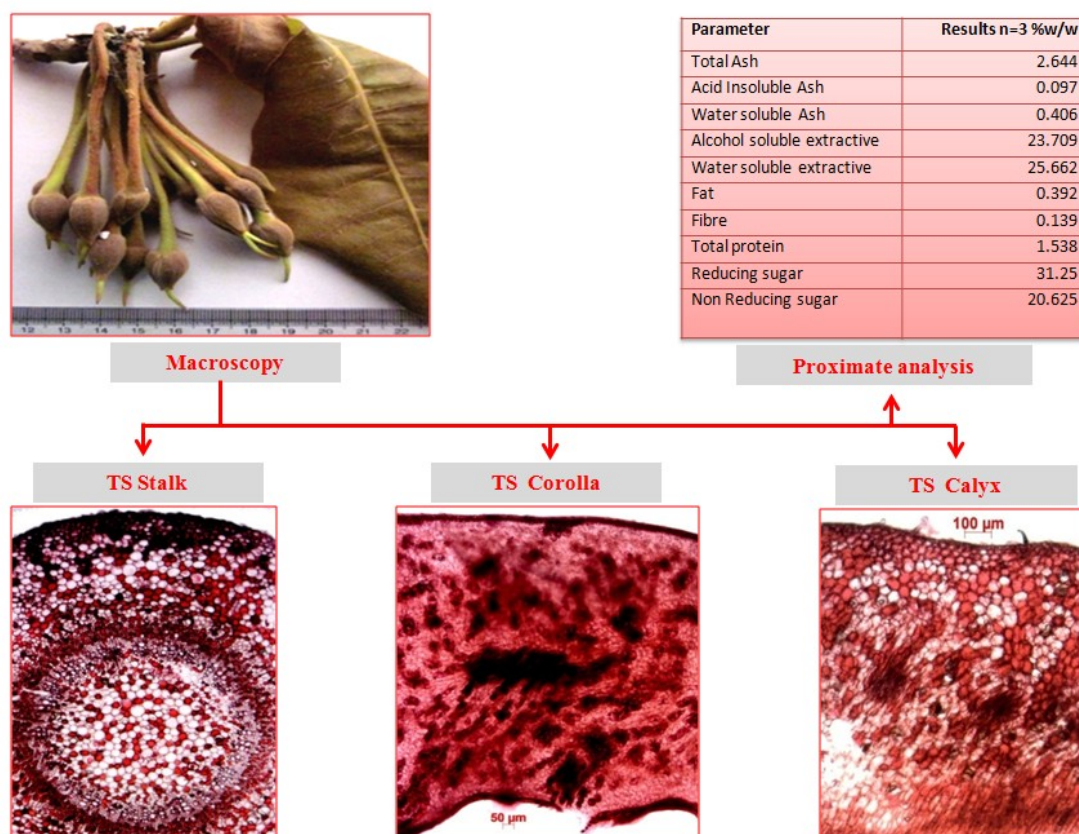
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## ABOUT FIRST/CORRESPONDING AUTHOR/S

**Dr. Juliet Elizabeth Castelino BAMS** is a final year PG scholar at Department of Dravyagauna, SDM College of Ayurveda Kuthpady, Udupi. Recently she has submitted her dissertation work to RGUHS Bangalore. Her field of interest are clinical practice with single drugs and new drug discovery.

**Dr. Suma V. Mallya MD, PhD (Ayu)** is working as associate professor at SDM College of Ayurveda, Kuthpady, Udupi. She is a recognized PG and PhD guide in Dravyagauna from RGUHS Bangalore. She has presented many papers in national and international seminars and published around 12 scientific papers in various journals.

## GRAPHICAL ABSTRACT



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