PREPARATION AND EVALUATION OF HERBAL FORMULATION GUDUCHI LEHYA

Mr. M. Samuel *, V. Yamini Seshasri, A. Uma Sai Naga Lakshmi, CH. Uday Bhaskar, P. Naga Vasudha, Dr. J. N. Suresh Kumar (principal)

Department of Pharmacognosy,
Narasaraopeta Institute of Pharmaceutical Sciences, Kottapakonda road, Yallamanda, Narasaropet, Guntur, Pin: 522601

ABSTRACT:

Guduchi (Tinospora Cordifolia Miers) is one of the widely used herbs for therapeutic purpose. In present study, Guduchi lehya which is used as immune booster is being prepared. For the preparation of Guduchi lehya, various physical examinations are examined. It has been proved beneficial and economical too.

KEYWORDS: Tinospora Cordifolia linn, lehya, avalehya, preparation methods, evaluation tests.

INTRODUCTION TO NATURAL SYSTEM OF MEDICINE:

From times immemorial, man is dependent on plant for his survival, the important necessities of life, food, clothing, shelter and medicines are supplied to a great extent by plant kingdom. The relationship between man and plants has been close throughout the development of human culture. Man’s eternal quest for happiness would continue till he draws his final breath. This quest has made him tread many paths known and unknown.

Disease has been an integral part of man from the beginning of his existence. The subject of drugs is also as old as disease and the search for remedies to combat it is perhaps equally old. The human being more afflicted by disease and he very early sought to alleviate his suffering from injury or disease taking advantage of plants growing around him. The plants “The sleeping giant of drug development” represent today the prime source of medicaments all over the world.
INTRODUCTION TO INDIAN SYSTEM OF MEDICINE:

Traditional system of medicine plays an important role in primary health care needs. The system of medicines which are of Indian origin or which have come to India from outside and got incorporated into Indian culture are known as Indian system of medicines. India has a rich heritage of traditional medicines and traditional health care system. In all traditional medicines, the medicinal plants play an important role. Indian traditional medicine based on different system of medicines which include Ayurveda, Yoga, Unani, Siddha and Homoeopathy.

Ayurveda

Ayurvedic System of medicine is the oldest medical system. Ayurveda word means “The science of life”. The Ayurveda concept was developed between 2500 and 500 B.C. in India. The word “Ayurveda” comprises of ‘Ayur’ (Life) and ‘Veda’ (Science). The Indian Hindu mythology states four Vedas written by the Aryans, which forms the backbone of Indian civilization. The four Vedas are: Rigveda, Samaveda, Yajurveda and Atharvanaveda. The Ayurveda is the part of Atharvanaveda.

Basic Principles of Ayurveda:

The basic principles of Ayurveda consist of:

a) Panchamahā Bhutas:-

According to Ayurveda the universe is made up of five basic elements also known as Panchama bhutas i.e. Akasa (Space), Vayu (Air), Teja or Agni (Fire), jala (Water), and Prithvi (Earth).

b) Trigunas:-

The three gunas:- Satva, Rajas and Tamas are the three essential components of the mind.

c) The Tridoshas:-

The three tridoshas are:- Vata(air), Pitta(bile) and Kapha(Phlegm).

d) The saptha dhatu:- Nourishment of these seven body tissues take place in sequential manner with original material provided by digested food material.
The saptha (seven), dhatus (tissues) elements for the pillars of the body. These are the means of nourishment and growth and also provide support to the body as well as the mind. These are:

e) The Trimalas:-

Malas are the various waste products of food and the dhatus produced during the normal digestive and metabolic process. The three primary malas are:

- Purisha (faeces)
- Mutra (urine)
- Sveda (sweat)

f) The Trayodosa Agni:-

It is a biological fire that controls metabolism. Agni balances all the changes in the body and mind like digestion and absorption of food, cellular transformations, assimilation of sensory perceptions and mental and emotional experiences. Agni covers entire sequences of chemical interactions and changes.

**GUDUCHI**

**Biological source:**

It consists of dried, matured leaves and pieces of stem of *Tinospora cordifolia Miers*.

**Family:** Menispermaceae

**Parts used:** Leaves or whole plant.

**Scientific classification:**

Kingdom- Plantae

Sub kingdom- Angiosperms

Super division- Eudicots

Orders- Ranunculates

Family- Menispermaceae

Genus- Tinospora

Species- *cordifolia*.
Vernacular Names:
Sanskrit- Amrita, Guduchi
Hindi- Giloy, Guruc, Gurcha.
Telugu- Tippateega.
Tamil- Shindiakodi

Geographical distribution:
*Tinospora cordifolia* indigenous to India, china, Myanmar, Sri Lanka, Thailand, Philippines, Indonesia, Malaysia, Bangladesh, north Africa, west Africa, and south Africa and also contains about 70 genera and 450 species. It typically grows in deciduous and dry forests at elevations up to 1000ft.

*Tinospora cordifolia* is a climbing shrub native to lower elevation in tropical areas of the Indian subcontinent and climbs numerous types of trees. It prefers wide range of soil, acid to alkaline and needs moderate level of soil moisture.

It is usually grows in tropical and dry areas and does not tolerate moist and humid climate. It can be grown under varying climate conditions. It is habitated throughout tropical India ascending to an altitude of 5600 m in the temperature range of 20 to 40°C. It thrives well in almost all types of soils. Sandy loam soil, rich in organic matter with good drainage is found to be good for higher yield of crop. Propagated by using stout stem cuttings. It is cultivated at onset of monsoon during May-June.

Botanical Distribution:

It is large, deciduous, extensively-spreading, climbing shrub with several elongated twining branches. Leaves are simple alternate, and estipulate with long petioles up to 15cm (6 in) long which are roundish and pulvinate, both at the base and apex with the basal one longer and twisted partially and half way around. It gets its name heart-leaved moonseed by its heart shaped leaves and its reddish fruit.
Morphology:

Plant description:

- Guduchi is perennial plant of weak and fleshy stem found throughout India.
- The aerial roots that arise from the stem are rainy season.
- The fruits of guduchi are pea like which are seen in water in India.
- A big climber (glabrous) climbs on large trees.
- Stems: Fleshy
- Roots: Long thread like, aerial, arise from branches. Bark thin, greyish or creamy white in colour, when peeled fleshy stem is exposed.
- Leaves: Cordate (pear shaped), Membranous juicy.
- Flowers: Bloom during summer.
- Male flower: Small, yellow or green coloured occur in clusters.
- Female flower: Singly.
- Fruits: Pea shaped, fleshy, shiny, turn red when boiled occurs in winter.
- Seeds: Curved pea sized.

Chemical Constituents:

Compounds like columbine, tinosporine, tinosporol, gilonin, syringing, cordifolioside A, tinosporidine, tinosporoside, tinosporaside, jatorrhizine, palmatine, tembeterine, tinocordifolioside, phenyl propene disaccharides, choline, tinosporic acid, bitter principles identified as chasmanthin and columbi. The stems are rich in proteins, starch, calcium and phosphorus, tinosporide have been isolated from *Tinospora cordifolia*.

LEHYA

Lehya is is one of the forms of ayurvedic medicine having semi-solid consistency. It is prepared from herbs by the addition of Gur (jaggery), Sharkar (sugar or sugar candy) and boiled with prescribed swarasa (drug juice) or Kwath / Kashayam (decoction).

AVALEHYA

- Avalehya is also known as jam or paste like product.
- Jaggery or sugar candy is dissolved in liquid, boiled and strained.
- The powdered drug in small quantities are added and stirred continuously to form homogenous mass.
• Ghee or oil is added when preparation is added hot.
  Ex: Chyawanprash, Kutajavaleha, Drakshavaleha.

METHOD OF PREPARATION OF LEHYA:

In the preparation of lehya ingredients present are:

A) Kashaya (decoctions or other liquids)

B) Gur / Guda / Sharkara (jaggery, sugar or sugar candy)

C) Churna (powders or pulps of certain drugs)

D) Ghrita (Ghee or tailam (oil)

E) Madhu (honey)

• Gur / Guda / Sharkara (jaggery, sugar or sugar candy) is dissolved well in the decoction or liquid and strained to remove the foreign particles. This solution is then boiled over a moderate fire.

• When the Pak (Phanita) is tantuvat (thread like) when pressed between thumb and index finger or when it sinks down in a glass of water without getting easily dissolved, it should be removed from the fire. Churna (fine powders) of herbs are then added in small quantities and stirred continuously and vigorously to form a homogenous mixture.

• Ghrita (Ghee) or Taila (oil), if mentioned is added while the preparation is somewhat hot and mixed well. Madhu (honey), if mentioned is added at the last when the mixture or preparation gets cool and mixed well.

DIFFERENT PARAMETERS OF STANDARDIZATION OF LEHYAS ARE:

1. Organoleptic parameters:

a) Color of sample

b) Odour of sample

c) Taste of sample

d) Determination of P^H of sample
Procedure:

1. Wash six test tubes with distilled water and put them on test tube stand and label them A, B, C, D, E, F.
2. Add 2 ml of CH3COOH in test tube A, add 2 ml of NaOH in test tube B, add 2ml of NaCl in test tube C, add 2 ml of NaHCO3 in test tube D, add 2 ml of water in test tube E, and add 2ml of lemon juice in test tube F.
3. Take white tile, place 6 pH paper and label them A, B, C, D, E, F.
4. Use a dropper or glass rod to put the respective sample solutions on the labelled pH paper placed on the white tile.\(^{(46)}\)
5. Observe the colour change.

Observation:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colour on PH paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Orange</td>
</tr>
<tr>
<td>B</td>
<td>Dark blue</td>
</tr>
<tr>
<td>C</td>
<td>Red</td>
</tr>
<tr>
<td>D</td>
<td>Light blue</td>
</tr>
<tr>
<td>E</td>
<td>Green</td>
</tr>
<tr>
<td>F</td>
<td>Pink</td>
</tr>
</tbody>
</table>

Table 4.1: Colour Observation
Result:

<table>
<thead>
<tr>
<th>Test tube</th>
<th>Solution</th>
<th>PH colour paper</th>
<th>pH</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>CH₃COOH</td>
<td>Orange</td>
<td>3</td>
<td>Weak acid</td>
</tr>
<tr>
<td>Sample B</td>
<td>NaOH</td>
<td>Dark blue</td>
<td>14</td>
<td>Strong base</td>
</tr>
<tr>
<td>Sample C</td>
<td>NaCl</td>
<td>Red</td>
<td>1</td>
<td>Strong acid</td>
</tr>
<tr>
<td>Sample D</td>
<td>NaHCO₃</td>
<td>Light blue</td>
<td>9</td>
<td>Weak base</td>
</tr>
<tr>
<td>Sample E</td>
<td>Water</td>
<td>Green</td>
<td>7</td>
<td>Neutral</td>
</tr>
<tr>
<td>Sample F</td>
<td>Lemon juice</td>
<td>Pink</td>
<td>2</td>
<td>Weak acid</td>
</tr>
</tbody>
</table>

Table: Result values

2. Physical parameters:

a) Determination of foreign organic matter

Procedure:

Weigh 100-500gmor the quantity specified in the individual monographs, of the original sample and spread it out in a thin layer. Inspect the sample with the unaided eye or with the use of a 6x lens and the separate the foreign organic matter manually as completely as possible. Weigh and determine the percentage of foreign organic matter from the weight of the drug taken. Use the maximum quantity and sample for coarse or bulky drugs.

c) Determination of Ash value
• **Total ash value**

Total Ash value is used to measure the total amount of material remaining after incineration.

1. Weigh accurately about 3gms of the powdered drug in a traced silica crucible.
2. Heat at 450 degree centigrade until free from carbon.
3. Cool and weigh.

\[
\% \text{ Total Ash} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100
\]

• **Acid insoluble ash**

Acid insoluble ash is the residue obtained after boiling the total ash with dilute HCl and igniting the remaining insoluble matter.

1. Total ash
2. Add 10 ml 3N HCl and boil for 5 min.
3. Filter through ash less filter paper
4. Ignite at 450°C
5. Cool and weigh.

\[
\% \text{ Acid insoluble ash} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of sample}} \times 100
\]

• **Water soluble ash and Sulphated ash**

Water soluble ash is the difference in weight between total ash and residue after treatment of total ash with water

1. Total ash
2. Add 10 ml water and boil for 5min
3. Filter through ash less filter paper
4. Ignite at 450°C
5. Cool and weigh.

Weight of water soluble ash=weight of total ash-weight of water insoluble ash
% water soluble ash = weight of water soluble ash / weight of sample x100.

c) Determination of extractive value, alcohol soluble extractive value, water soluble extractive value.

Procedure:

The powder was packed in filter paper thimble and extracted with petroleum ether (60-80 degree centigrade) in the soxhlet apparatus for 16 hours till all the fatty substance and others were extracted. The completion of was checked by taking a spot of petroleum ether on the silica gel plate (TLC plate), on developing in iodine chamber which should not develop any colour.

The extract thus obtained was concentrated by distilling off the solvent under reduced pressure. The defatted marc thus obtained was successively extracted with chloroform methanol. The extracts were concentrated separately under reduced pressure.

**Showing extractive values of different solvents:**

<table>
<thead>
<tr>
<th>EXTRACT</th>
<th>COLOUR</th>
<th>EXTRACTIVE VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Petroleum ether</td>
<td>Green</td>
<td>1.04%</td>
</tr>
<tr>
<td>2. Chloroform</td>
<td>Dark green</td>
<td>1.60%</td>
</tr>
<tr>
<td>3. Alcohol</td>
<td>Dark brown</td>
<td>10.16%</td>
</tr>
</tbody>
</table>

**Table: Extractive values**

d) Loss on drying

A watch glass is weighed accurately and then 1gm of sample is taken in weighed watch glass and dried in an electric hot air oven at 1100 degree centigrade for 6 hours. After that is cooled and again weighed. The difference in the two weights gives loss on drying of the sample in percentage.

e) Determination of specific gravity
Procedure:

- Clean and dry the density bottle.
- Wash the bottle with water and allow it to drain.
- Wash it the alcohol and drain it to remove water.
- Wash it with alcohol and drain it to remove water.
- Wash it with ether, to remove alcohol and drain water.
- Weigh the empty bottle with stopper (W1)
- Take about 10 to 20gm of oven soil sample which is cooled in a desiccator and transfer it to the bottle. Find the weight of the bottle and soil (W2).
- Put 10ml of distilled water in the bottle to allow the soil to soak completely. Leave it for about 2 hours.
- Again fill the bottle completely with distilled water put the stopper and keep the bottle under constant temperature water baths (Tx0).
- Take the bottle outside and wipe it clean and dry note. Now determine the weight of the bottle and the contents (W3).
- Now empty the bottle and thoroughly clean it. Fill the bottle with only distilled water and weigh it. Let it be 24 at temperature (Tx0C).
- Repeat the same process for 2 to 3 times, to take the average reading of it.

f) Determination of solid content

Procedure:

Total solids (TS)

- Ignite a clean evaporating dish at 550 c in a muffle fumance for 1 hr.
- Cool the dish, weigh and keep it in a desiccator.
- Transfer carefully 50ml of sample into the dish and evaporate to dryness on a steam bath.
- Place the evaporated sample in an oven adjusted at 103 0c and dry it for 1 hr.
- Repeat drying at 103°c till constant weight is obtained.
Total volatile solids:

- Ignite the above dish with residue at 500 °C for 15 mins.
- Allow the dish to cool partially in air aid place and transfer to a dessicator.
- Weight till constant weight is obtained.

g) Determination of alcohol content

**Procedure:**

Method followed:

1. **For liquid presumed to contain less than 30% v/v alcohol**
   - Take 25 ml sample, add equal volume of water and distilled.
   - Collect distillate 2 ml less than the original volume (23 ml).
   - Add water to make it 25 ml.
   - Adjust the temperature and find specific gravity or refractive index.
   - From alcohol metric table find the % age of alcohol.

2. **For liquid presumed to contain more than 30% v/v alcohol**
   - Take 25 ml sample add 50 ml water and distilled.
   - Collect distillate 2 ml less than 50 ml (48 ml).
   - Add water to make it 50 ml.
   - Adjust temperature and find specific gravity or refractive index.
   - From alcohol metric table find the %age of alcohol.

h) TLC-Fp techniques

Aliquots of 2 μL of propolis extracts were applied to the 20 × 10 cm silica gel HPTLC plates (Art. 105641, Merck) as 8 mm band by using Automatic TLC sampler 4 (ATS4, CAMAG, Muttenz, Switzerland). Plates were developed with a mixture of toluene–ethyl acetate–formic acid (6 : 5 : 1, v/v/v) in the saturated (20 min) twin trough chamber up to the distance of 70 mm. Developed plates were dried for 5 min with a hairdryer.

The plates were than heated for 3 min at 100°C on TLC Plate Heater III (CAMAG) and immediately dipped in 0.5% solution of NTS in ethyl acetate for 1 s, by using Chromatogram Immersion Device III (CAMAG). After 5 min of drying in the air, the plates were immersed in 5% solution of PEG 400 in dichloromethane for 1 s, for enhancement and stabilization of fluorescent zones. Images were captured at 366 nm with DigiStore 2 device.
image analyzing system in conjunction with Reprostar 3 (CAMAG). Four apertures with exposure time of 30 ms and frame of −2 mm were applied. The photos were stored as TIF files for further image processing.

- When pressed between fingers, shows thread like stretched.
- Sinks in water without getting easily dissolved.
- Finger leaves impression on it.

**Results**

Standardization of guduchi *lehya* was carried out based on the physicochemical parameters. Three commercial preparations of *guduchi lehya*, were found to pass most of the physicochemical tests. However, some of the physicochemical parameters of were found to be quite different. Guduchi lehya constitutes of 4 ingredients namely *Tinospora cardifolia Miers*, *Cuminum Cyminum*, *Apis mellifera* and *Elettaria cardamomum*.

**CONCLUSION**

Although Ayurveda advocates the use of quality control tests to make sure that the prepared medicines adhere to the standards mentioned in Ayurveda, most of the tests described appear to be based on observation and seem subjective without valid scientific backing. Hence, standardization and development of reliable quality protocols for Ayurvedic formulations using modern techniques of analysis is extremely important. Since standardization and development of reliable protocols for quality control of Ayurvedic formulations using modern techniques of analysis are extremely important, the generated TLC fingerprints of the n- hexane and the acetone extracts of Guduchi lehya could be used as a valuable analytical tool in the routine standardization of Guduchi lehya to check the batch to batch variations.