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DEVELOPMENT OF PHARMACOGENETIC STANDARDS OF THE DECOCTION OF SHIN MIDI (*PREMNA INTEGRIFOLIA*) GROWN IN SRI LANKA

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ABSTRACT

Sihin Midi (*Premna integrifolia*, Family – Verbenaceae) also known as *Agnimantha* in Ayurveda texts. It is a large shrub and has vata kapha shamaka properties. This plant has been used for a wide variety of diseases in Ayurveda and Traditional Medicine in Sri Lanka. **Aim of Study:** This study was aim to establish pharmacogenetic standards of the decoction of *Shin Midi*. **Materials and Method:** Organoleptic properties and microscopical identification of dried roots of *Sihin Midi* powder were studied. Determination of loss on drying, total ash, acid insoluble ash, water insoluble ash, water soluble extractive and acid soluble extractive values were calculated as per the Ayurveda Pharmacopeia of India. **Results:** *Sihin Midi* roots were woody branched, yellowish brown in color and somewhat tortuous to cylindrical in shape. Microscopically observed the presence of characteristic oil globules in the cortical region, prismatic crystals of calcium oxalate were seen in the cortical cells. n-Hexane hot extraction of *Sihin Midi* roots showed the positive reaction for alkaloids, tannins, saponin, glycosides and flavonoids. TLC profile of n-Hexane extractions of roots powder of *Sihin Midi* shows UV active zones at 254 nm and 366 nm. **Conclusion:** The findings from this study facilitated the identification of proper dried raw materials for the preparation of decoction. The Phytochemical analysis of dried roots of *Sihin Midi* indicted the presence of alkaloids, tannins, glycosides and flavonoids which have potential role in medicinal value. It suggests for further investigation and isolation of biologically active constituents responsible for the obesity.

KEYWORDS: *Premna integrifolia*, *Sihin Midi*, pharmacogenetic standards, phytochemicals.

INTRODUCTION

Sihin Midi is a local name of *Agnimantha* which has been used, as a medicine from Vedic period, having evidences of various pharmacological properties. *Sihin Midi* plant commonly known as *Heen Midi* or *Midi Gas* in clinical practice in Sri Lanka. According to the Wealth of India, *Premna integrifolia* is the botanical source of *Agnimantha* plant mention in Ayurveda. *Sihin Midi* has been identified as *Premna integrifolia* Linn. of verbenacea family and its synonyms, *Premna serratifolia* Linn.[1] *P. obtusufolia* R. Br and *P. corymbosa* Acut.[2] It is a large shrub having fruits looks like small grapes and it distributed throughout Asia. The leaves, wood and roots of the plant had been used as a medicine for a wide variety of diseases. The root of *Agnimantha* is one of the ingredients of the famous Ayurvedic medicine *'dashamula'*.[3] In Susrutha Samhitha, *Agnimantha* had been mentioned as one among *Vruhath Panchamula*.[4] Pharmacognostic identification of *Sihin Midi*/ *Agnimantha* is argument among the Ayurveda and Traditional physicians in Sri Lanka. Therefore, herbal industries and local communities generally face the problems of the identification and authentication of the raw materials of *Sihin Midi*/*Agnimantha* from adulterants and substitution. The aim of the present study was to establish pharmacogenetic standards including phytochemical and physico-chemical analysis of the decoction of *Shin Midi* (*Premna integrifolia).* The present authenticate it based on taxonomic and pharmacognostic analyses.

Vernacular names of *Sihin Midi*

The vernacular names are the different names of the drug in different languages. This will identify the particular drug at different areas. By knowing the names of the drugs in the particular area, can get knowledge about the plant in its various aspects.

Sinhala name - *Sihin Midi, Heen Midi, Middee Gas, Maha Midi, Wal-Wel- Midi, Midi*[2]
English name - Egh wood, Headache tree[2]
Sanskrit name - *Agnimantha*
Hindi name - *Arani, Agethu, Ustabunda, Ganiyar, Bakar, Gin*
Tamil name - *Munnay, Muney, Kiray*[3]
Marathi - *Eran, Takli*
Gujarati - *Arni*
Assame - *Gainali, Gonderi, Gunaru, Gaimali, Gunarua*

Kannada - *Agnimantha*

According to Encyclopedic dictionary of Ayurveda, the etymological way the technical term for the plant has arrived signifies its characteristics. The Greek name of *Premna* was '*Premnon*' means the trunk or a stump of a tree, referring to the dwarf stature of one species. '*integri'* means entire or whole and '*folia*' means leaves. In the synonyms, *serratifolia* is from the Latin word '*serratus*' means saw-toothed leaves.[5]

The *Agnimantha* word was not found in Rig-Veda. However, in Vedic period *Agnimantha* wood used to produce fire by friction. The word "*Sraktya*" which was Sanskrit name of *Arani* (synonym of *Agnimantha*) was available in the Atharvaveda (AV.II. 4.4 & AV. II 11.2).[6]

Leaves

Leaves are opposite, light green in colour with prominent veins and the mid rib rose beneath. Leaves have an unpleasant odour.[2] Leaves are 5.0 - 9.0 cm by 3.2 - 6.3 cm, broadly elliptic, obtuse, very shortly acuminate, glabrous, entire or the upper part dentate, base rounded or sub-acute; main nerves 4.0 – 5.0 pairs; petioles 1.0-1.6 cm long.[1] The leaves are oval, acute or rounded at the base, acute or sub-acute at the apex, sometimes whorled, elliptic, ovate membranes when young, coriaceous when mature, entire or irregular.[7]

Flowers

Flowers are bisexual, small greenish white on short pubescent pedicles with a strong disagreeable odour.[7] The small, numerous flowers are cream-green in colour, with rather an unpleasant odour, borne on spreading terminal pubescent paniculate corymbose cymes about 10.0 - 20.0 cm across. Usually Flowers are appearing in March and September.[8] Bract is minute, lanceolate. Calyx is 2.5 mm long, thick, glabrous, two lipped, and one lip two toothed, the other subentire. So that calyx appears three lobed. Corolla is glabrous outside; tube is 3.0 by 2.0 mm, cylindrical, hairy in the throat inside, lobes four, oblong, rounded, 1.2 mm long. Stamens slightly exerted. Filament is hairy at the base. Ovary and style are glabrous. Stigma has two equal divaricated lobes.

Fruits

Fruits are 4.0 mm long, pear shaped; endocarp ridged, bony, four celled and four seeded.[1] Fruits are globose drupes, normally 4.0 chambered, dark blue to black when mature and stone hard. The plants propagated from seed.[8]

Wood

Wood is whitish with purple streaks or light creamy brown to pale grayish white, pleasantly scented, moderately hard.

Roots

Roots are light brown or yellowish-brown woody, aromatic.[1]

Habitat and Distribution

P. integrifolia grows along the seacoasts of Sri Lanka, Bangladesh to Thailand, southern China, Seashells, Madagascar, India, Andamans and Nicobaras.[9]

Parts used in medicine

Generally, roots and leaves used for the treatment in Ayurveda medicine.[10]

Dosage forms of *Sihin Midi* (*Agnimantha*)

Choorna – 3.0 g, *Swarasa*– 10.0– 20.0 ml, *Kwatha* –10.0 – 100.0 ml.

***Sihin Midi* included drug formulation**

Dashamula kvatha, Dashamula arishta, Dashamula churna, Agnimantha mula kalka, Agnimantha kashaya, Narayana tailaya, Chavaanaprashavalehaya, Ahiphenasavaya, Karpura rasa, Nidrodaya vati, Kamini vidravana rasa, Mushakadya tailaya, Vajra kapota rasa.

According to Ayurveda[11] *Sihin Midi/Agnimantha* having *Katu, Thiktha, Kasaya* rasa, *Ruksha, Sukshma* guna, *Ushna* veerya and *Katu* vipaka.

Specific actions

Deepana (appetizer), *pachana* (stimulate digestion), *anulomana* (carminative), *medo rogahara* (alleviating fat disorders in the body), *kapha vata nasaka* (pacify *kapha* and *vatha dosha), shothahara* (aleviating oedema), *vedana sthapana* (pain killer), *raktha shodaka* (purify of blood), *hriduttejaka* (heart stimulating), *pramehaghna* (alleviating conditions of polyurea and abnormal urine).[2,11]

Phytochemicals and Pharmacological study of *P. integrifolia*

Wealth of India has been described that Premnine, Ganiarine and Ganikarine are three main active principles present in *P. integrifolia.* Roots of the plant have premnine, beta-sitosterol,

triterpenes, polyphenols, n-octacosanol and n-triacontanol along with some inorganic salts. The roots contain a yellow coloring matter, tannin and an essential oil which had used in Sri Lanka for the treatment of colic. An antibiotic of phenolic nature had been isolated from the fresh root bark. It had found to be active against the Gram – positive organisms.[1,2] *Premna serratifolia* Linn. did not produce any toxic symptoms nor mortality up to the dose level of 2000.0 mg / kg body weight in rats, and hence the extract was considered to be safe and non-toxic. Root bark of *Agnimantha* (*Premna obtusifolia* Linn) shows significant therapeutic value in obesity, showed remarkable decrease in BMI, triglyceride, cholesterol -HDL ratio, LDL - HDL ratio after nine months of treatment and no adverse effect of the drug has been recorded.[12] Moreover, there are several reports,[13] on a study of *Shilajeet* with *Agnimantha Swarasa* in the management of *Medoroga*, the effect of *Agnimantha Quawath Bhavita Shilajeet* on *Medoroga*[14] and *Gomutra Arka Bhavita Agnimantha* for *Sthaulya*.[15] The effect of *Premna integrifolia* Linn (Verbenaceae) on blood in Streptozotocin induced type1 and type II diabetic rats also reported.[16]

MATERIALS AND METHODS

Authentication and Processing of the raw materials

Fresh roots and areal parts with flowers of *Sihin Midi* were collected from Putlam district in Sri Lanka (GPS 8° 2' 0" N 79 ° 49' 0"E), in July 2014. Plant materials were identified and authenticated by a botanist at National Herbarium at Peradeniya, Sri Lanka. After removal of all foreign matters of roots, were washed with running water then was cut in to small pieces and was dried under shade conditions for 30 days to avoid chemical changes. The dried samples were coarsely powdered using grinding machine, was stored in polythene bags at room temperature and was used for trial drug and extractions in standardization procedures. Coarsely powdered 60.0 g of *Sihin Midi* roots and 1920.0 ml of water was added to earthen pot and was boiled over moderate fire until reduced to 240.0 ml of the volume.This gives the conventional dose for an adult which is 240.0 ml per day.

Pharmacogenetic study of dried roots of *Sihin Midi*

Root samples were identified and were authenticated by the Department of Dravya Guna Vingnana, Institute of Indigenous Medicine, University of Colombo, Sri Lanka. The root samples of *Sihin Midi* were pharmacognostically identified from the Herbal Technology Section, Industrial Technology Institute, Sri Lanka.

Identification of macroscopic and microscopic characters of dried roots of *Sihin Midi*

Dried roots of *Sihin Midi* powder organoleptic characteristics such as appearance, colour and odour were studied. The dried root samples of *Sihin Midi* were macroscopically and microscopically identified from Herbal Technology Section, Industrial Technology Institute according to WHO guidelines.

Physico-chemical analysis of dried roots of *Sihin Midi*

Determination of loss on drying, total ash, acid insoluble ash, water insoluble ash, water soluble extractive and acid soluble extractive values were calculated as per The Ayurvedic pharmacopeia of India.

Determination of loss on drying

The root powder of the *Sihin Midi* 2.0 g accurately weighed and taken in a watch glass previously weighed and dried in an oven at 105° C until constant weights were achieved. The weight after drying was noted and the loss on drying was calculated. Then the percentage was calculated.

$$\text{Percentage of loss on drying} = \frac{\text{Loss drying}}{\text{Weight of sample}} \times 100$$

Determination of total ash, water soluble ash and acid insoluble ash

The root powder of the *Sihin Midi* 3.0 g accurately weighed and taken in to a porcelain crucible and was incinerated in a muffle furnace at a temperature not exceeding 450°C for about four hours until it is white, then was left it for cool in desiccator for 30.0 minutes and weighed. The total ash value was calculated in percentage.

$$\text{Percentage of total ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of acid insoluble ash

The root powder of the *Sihin Midi* 3.0 g accurately weighed and was taken in to a Silica crucible and was incinerated in a muffle furnace at a temperature not exceeding 450°C for four hours then was left it for cool and weighed. The ash of the drugs obtained was boiled with 25.0 ml of 2.0 N HCl acids for five minutes and then solution was filtered through Whatman filter paper No.41. Then acid insoluble particles in ash were washed with hot water until the filtrate was neutral. Insoluble ash was transferred to crucible, was dried in oven to constant weight. Then it was left it for cool in desiccator for 30.0 minutes and was weighed. The percentage of acid insoluble ash was calculated.

$$\text{Percentage of acid insoluble ash} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of sample}} \times 100$$

Determination of water-soluble ash

The root powder of the *Sihin Midi* 3.0 g was weighed and was taken in to a Silica crucible and was incinerated in a muffle furnace at a temperature not exceeding 450° C for about four hours, then was left it for cool. 25.0 ml of water was added to the ash and was boiled for five minutes. Then it was transferred insoluble ash to crucible, was dried in oven to constant weight and then was left it for cool in desiccator for 30.0 minutes. Insoluble ash particles were weighed. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air dried sample of the drug.

$$\text{Percentage of water-soluble ash} = \frac{\text{Weight of water-soluble ash}}{\text{Weight of sample}} \times 100$$

Determination of extractability

Water-soluble extract

Root powder of *Sihin Midi* 5.0 g of was taken in to a conical flask. 100.0 ml of distilled water was added shake it, and was kept overnight. Next day it was filtered and the filtrate was used for further experiment. 20.0 ml of the filtrate was taken in to a weighed, dried porcelain evaporating dish and was evaporated using a hot water bath. It was dried to constant weight in an oven and was weighed. From the weight of the residue, the water-soluble extractive percentage was calculated.

Methanol Soluble Extractive

Sihin Midi root powder 5.0 g was taken in to a conical flask. 100.0 ml of Methanol was added shake it, and was kept overnight. Next day it was filtered and the filtrate was used further in the experiment. The filtrate 20.0 ml was taken in to a weighed, dried porcelain evaporating dish and was evaporated using a hot water bath. It was dried to constant weight in an oven and was weighed. From the weight of the residue, the Methanol soluble extractive percentage was calculated.

Phytochemical analysis of dried roots of *Sihin Midi*

Hexane extract was prepared from 5.0 g of coarsely powdered roots of *Sihin Midi* using the Soxhlet apparatus. It has been subjected to preliminary qualitative phytochemical screening

for identifying the presence of various phytoconstituents like alkaloid, tannins, saponins and flavonoids.

Test for alkaloids

Three drops of dilute HCl acid was added to 2.0 ml of extraction and a few drops of Mayer's regent was added and change was noted.

Test for tannins

A few drops of Ferric chloride regent were added to the extraction and the change was noted.

Test for saponins

The extraction 1.0 ml was shaken vigorously with 2.0 ml of distilled water in test tube for 30 seconds. Then the test tube was left to stand for twenty minutes. Formation of persistent frothing was noted.

Test for glycosides

Liebermann's Test. The extraction 1.0 ml was added 2.0 ml of acetic acid and 2.0 ml of chloroform. The mixture was then cooled and we added H_2SO_4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Test for flavonoids

Powder 1.0 g was extracted with 10.0 ml of 95 % Ethanol for 15 minuteson a boiling water bath. Small pieces of metal Magnesium and a few drops of concentrated HCl acid was added to the filtrate. The change was noted.

Thin Layer Chromatography (TLC) Fingerprints of *Sihin Midi*

The hot extraction and the cold extraction of the *Sihin Midi* roots powder and decoction of the *Sihin Midi* were spotted at the origin of three TLC plates. They were run in the solvent system of Hexane and Ethyl acetate, in the ratio of 7:3 in the TLC chamber. Detection was done by spraying with Vanillin sulphuric acid followed by heating at 100^0 C for 10 minutes. The number of spots, their Rf values and color recorded from the chromatographs of the samples.

RESULTS AND DISCUSSION

Two varieties of *Agnimantha* plant (*Agnimantha* and *Tarkari*) had mentioned in great Ayurveda texts of Vruhattrayi. *Bhawa Prakasha* had mentioned only one variety. Two

varieties (*lagu* and *vruhat*) of *Agnimatha* had described in *Nighantu Rathnakara***.** Further, it had mentioned that *laghu* variety had better *sothahara* (anti-inflammatory) property than the *vruhat* variety. *P. integrifolia* and *P. Latifolia* Var. *mucronata* Clarke (only found in India) had been botanically identified as *Agnimantha*. Further *Clerodendron phlomidis* plant had been identified as *Tharkari* and their properties also considered as similar. Most of the nighantu had considered that *Agnimantha* and *Tarakari* are different plants and botanically identified as *P. integrifolia* and *Clerodendron phlomidis* respectively.[17]

Pharmacogenetic study of dried roots of *Sihin Midi*

Organoleptic characteristics of roots of *Sihin Midi*

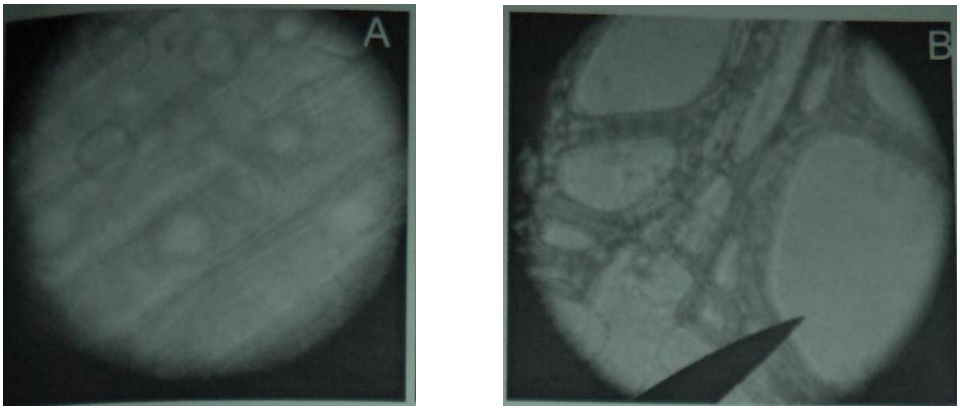
Sihin Midi roots were woody branched, yellowish brown in color and somewhat tortuous to cylindrical in shape. Surface of roots exfoliated easily, showed longitudinal striations and wrinkles (Fig.1). Roots were possessed slightly aromatic odour. Powder of roots was brown in colour having bland taste and slight aromatic odour (Fig. 2).

Fig. 1 Fresh roots and dried roots of *Sihin Midi.*

Fig. 2. Powder of dried roots of *Sihin Midi*

Microscopic characters of roots of *Sihin Midi*

Sihin Midi roots were identified microscopically according to World Health Organization criteria. Results can be summarized as, presence of characteristic oil globules in the cortical region, uniseriatee, modularly rays, large xylem vessels with bordered pits and prismatic crystals of calcium oxalate were seen in the cortical cells shown in the Fig.3 and 4.



A **B**

Fig. 3: Microscopical characters of *Sihin Midi* roots.

A. Cortical region showing characteristic oil globules under x 40
B. Xylum region showing uniseriate medullary rays and large xylum vessels under x 40

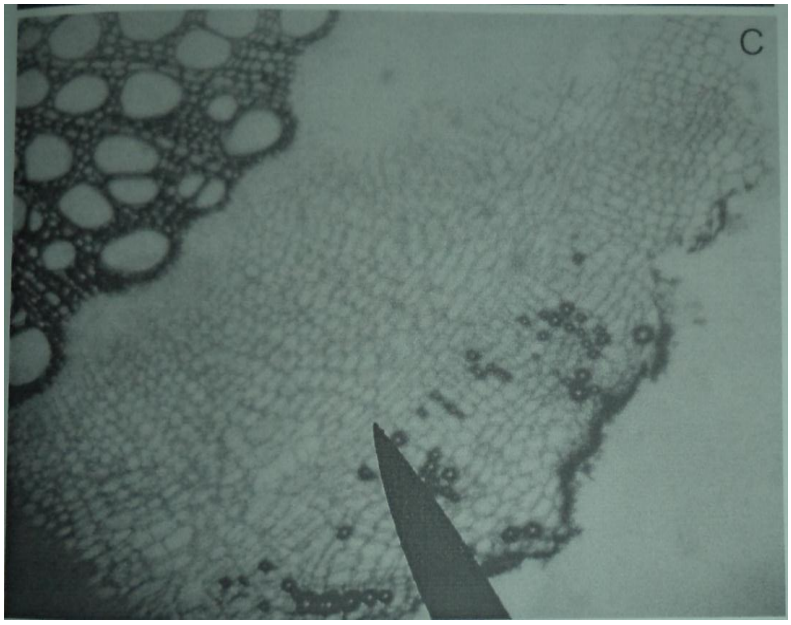


Fig. 4: Transverse section of the root of *P. integrifolia* (*Sihin Midi*) under x 10 of the compound microscope.

Physico chemical analysis of dried roots of *Sihin Midi*

The total ash of the dried roots powder of *Sihin Midi* was found 8.7 %, acid insoluble ash was found 8.1% and water insoluble ash was found 0.77%. The extractability in water and methanol were found 7.4% and 8.6% respectively. (Table 1)

Table 1: Ash and extractive values.

	Ash values (Mean)				Extractive values (Mean)	
	Loss on Drying	**Total Ash value**	**Acid Insoluble Ash**	**Water Soluble Ash**	**Water Soluble Extract**	**Methanol Soluble Extract**
Dried powder	6 % w/w	8.7 % w/w	8.1%w/w	0.77% w/w	7.4% w/w	8.6 % w /w

Preliminary phytochemical analysis of dried roots of *Sihin Midi*

Preliminary phytochemical analysis of the n-Hexane hot extraction of *Sihin Midi* roots showed the positive reaction for alkaloids, tannins, saponin and flavonoids (Table 2)

Table 2: Presence of phyto constituents of *Sihin Midi* decoction.

Classes of chemical compounds	Test/Reagent	Observations
Alkaloids	Mayer's Reagent	+
	Dragondroff Reagent	+
Glycosides	Liebermann's test	-
Flavonoids	Ortho - cyanidin test	-
Tannins	Ferric chloride solution test	+
Saponins	Frothing Test	+

Thin Layer Chromatographic identification of *Sihin Midi* root extractions

Thin layer Chromatography of n-Hexane extractions of roots powder of *Sihin Midi* shows UV active zones at 254 nm and 366 nm (Figure 4 .5)

CONCLUSION

In conclusion, pharmacogenetic standards were established for dried roots and decoction of *Shin Midi* (*P. integrifloria*) decoction for the first time in Sri Lanka. The findings from this study facilitated the identification of proper dried raw materials for the preparation of decoction. The Phytochemical analysis of dried roots of *Sihin Midi* indicted the presence of alkaloids, tannins, sugars and flavonoids which have potential role in medicinal value. It suggests for further investigation and isolation of biologically active constituents responsible for the obesity.

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REFERENCES

1. Kirtikar KR and Basu BD.2005, Indian medicinal plants. Vol III., Periodical Experts Book Agency, Vivek Vihar, New Delhi India, 2005; 32.
2. Senarathna LK.Check list of the flowering plants of Sri Lanka, National Science foundation, Colombo, Sri Lanka, 2001; 368.
3. Anonymous. Wealth of India, Vol VIII., Council of scientific and industial research, New Delhi, 1998; 240.
4. Sharma PV. Susruta Samhitha Volume 1, Suthra sthana 38/68, Chawkhambha Visvabharati, Varanasi, India, 2013; 351.
5. Lochan K, and Byadgi PS. Encyclopedic dictionary of Ayurveda. Chawkhamba publications, New Delhi, India, 2011; 15.
6. Ahmed SR, and Siddiq M. Vedic plants medicinal and other use. Chawkhamba Orientalia, Varanasi, India, 2009; 51.
7. Jayaweera DMA. 2006, Medicinal plants used in Ceylon. part V, National Science Council, Colombo, Sri Lanka, 2006; 173.
8. Dasanayake MD.A Revised handbook of the Flora of Ceylon. Vol. IV, Oxford and IBH publishing Co, Bombay, 1983; 316.
9. Nadkarni KM.1982, Indian Materia Medica, Vol 1, Popular Prakashan, India: pp.1009.
10. Chopra RN, and Chopra I C, Verma BS. "Glossary of Indian Medicinal Plants," CSIR, New Delhi, 1969.
11. Sharma PV. Dravya Guna Vignana. Vol 5, Chaukamba Barathi Academy, Varanasi, India, 2006; 5.
12. Golosh R., Golosh S, Maity L N. 2009, Therapeutic efficacy of *Agnimantha (Premna obtusifolia* Linn) in obesity (*sthaulya*). Indian J Tradit Know, 2009; 8(3): 369-371.
13. Tivari K K. A differential study of *Shilajeet* with *Agnimanth swarasa* and its *ghana* in the management of *Medoroga*. Department of Rasashastra Bhaishajya, Jamnagar, India, 1983.
14. Murlikrishna V. The effect of *Agnimantha* quawath bhavita shilajeet on *Medoroga* University of Hyderabad India, 1991.

15. Shah C. *Gomuthra arka bhavita Agnimantha* for *Sthaulya*, University of Jamnagar, Gujarat India, 2004.
16. Alamgir M, Rokeya B, Hannan JM. The effect of *Premna integrifolia* Linn (Verbenaceae) on blood in Streptozotocin induced type 1 and type 2 diabetic rats, University of Jahangimagar, Bangladesh, 2004.
17. Sharma PV. *Dravya Guna Vignana*. Vol 2, Chaukamba Barathi Academy, Varanasi, India, 2006; 2: 21-226.