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Cover story

Kampillaka

***Mallotus phillipinensis* (Lam.) Muell. Arg.**
Family: Euphorbiaceae
Vernacular names: Sinhala: *Hamparila*; **English:** *Kamala*; **Tamil:** *Kapila*; **Sanskrit:** *Kampilya, Kampillaka, Raktaphala, Recana*[1].

Mallotus phillipinens is a very common perennial shrub, one of the medicinally important plant used in indigenous system. *M. philippinensi*s has a widespread natural distribution, from the western Himalayas, through India, Sri Lanka, to southern China, and throughout Malesia to Australia. Trees are small to medium-sized monoecious in nature, up to 25 m tall and with a bole up to 50cm in diameter, but usually much less in number. Slash turning deep red. Branchlets are reddish- brown glandular. Leaves are alternate and simple, more or less leathery, ovate to lanceolate, cuneate to rounded with two glands at base. Leaves are mostly acute or acuminate at apex, conspicu- ously 3-nerved, hairy and reddish glandular beneath, petiole size 1–4cm long, puberulous and reddish-brown in color.

Male flowers in terminal and axillary position, 2–10cm long, solitary or fascicled paniculates spikes, each flower are with numerous stamens, small; female flowers have spikes or slender racemes, each flower with a stellate hairy, 3 celled ovary with 3 papillose stigmas. Fruit is a depressed-globose; 3-lobed capsule; 5, 7 mm, and 10 mm; stel- late; puberulous; with abundant orange or reddish glandular granules; 3-seeded. Seeds are subglobose and black in color and 4 mm across [2]. Major phytochemicals present in this genus contain different natural compounds, mainly phenols, diterpenoids, steroids, flavonoids, cardenolides, triterpenoids, coumarin, isocoumarins, and many more.

Specially roots, fruits and fruit powder (glands/hairs of the fruit) and the leaves are used for medicinal purposes. Leaves are bitter, cooling and appetizer. The glands/hairs of the fruit and the leaves[3]are recommended for dermal problems. Many scientific investigations have been carried out to validate and investigate the pharmacological activities of *M. philippinensis*.

The review paper of *Mallotus phillipinensis* (Lam.) Muell. Arg.on page 364.

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Cover story by Dr. H.G.S.P. Hewageegana and Dr. L.D.A.M. Arawwawala
Photograph by Dr. H.G.S.P. Hewageegana

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Contents

Appraisal and *in-vitro* study on anthelmintic effect of *Vernonia cinerea* (*Monerakudumbiya)* against larvae of *Haemonchus contortus* and *Toxocara canis*

E.R.H.S.S. Ediriweera[1]*, R. P. V. J. Rajapakse[2], W. D. Ratnasooriya[3]

Abstract

This study is an attempt to gather and preserve the knowledge on therapeutic effects of *Vernonia cinerea* (Family: Asteraceae; Sinhala name: *Monerakudumbiya*, Sanskrit name: *Sahadevi*) and to observe anthelmintic effect *in-vitro* as it is used in treatment of worm infestation by villagers. Information was gathered from Ayurveda and Sri Lankan traditional medical texts, traditional physicians, journals and web search. Extracted juice of fresh leaves, herbal gruel and decoction prepared from *V. cinerea* are administered orally to treat stomach-ache, diarrhoea, dysentery, haemorrhoids, jaundice, hepatitis, worm infections, tonsillitis, cough, fever, filariasis, malaria, wounds, snake bites, skin diseases, eczema, leprosy, painful urination and colic, urinary calculi, urinary incontinence in children, arthritis, to increase menstrual flow, to stimulate labour and expedite the expulsion of placenta and in veterinary practice. Anti-inflammatory, antipyretic, anti-diuretic, antihyperglycemic**,** antioxidant, antimetastatic, antitumor, antifungal, bactericidal, nephrocurative, nephroprotective, and hepatoprotective activities of *V. cinerea* are scientifically proven. *V. cinerea* is used in treatment of worm infections in humans. *In vitro* larvae migratory inhibition assay carried out by the authors with larvae of *Toxocara canis* and *Haemonchus contortus* using water extracts of *V. cinerea* has revealed 89.42% and 86.67% inhibition respectively. *Vernonia cinerea* (*Monerakudumbiya*) is a plant with highly diverse medicinal values and is effective in inhibiting larval migration of *Toxocara canis* and *Haemonchus contortus*.

Keywords: *Vernonia cinerea, Monerakudumbiya, Toxocara canis, Haemonchus contortus,*

Introduction

Vernonia cinerea (Family: Asteraceae; Sinhala name: *Monerakudumbiya*; Sanskrit name: *Sahadevi*) is a common weed with valuable medicinal properties. *Sahadevi* (*V. cinerea*) is a well-known plant; the reference regarding this drug could be traced out in Vedas. The word *Sahadevi* is available in the literatures of Vedic period like Atharvaveda, Samhitha and Garuda purana. Atharvaveda praised *Sahadevi* as *Arundhati, Visvarupa, Subhaga* and *Jivala*[1]. Medicinal values of herb *V. cinerea* were known to people since ancient days. *V. cinerea* is used to treat various ailments including worm infections by Sri Lankan Traditional physicians. Larvae (immature worms) of dog round worm (*Toxocara canis*) causes toxocariasis in humans. *Haemonchus contortus* is one of the nematodes that is responsible for anemia and death of infected goats. Infections of *Haemonchus contortus* in humans are very rarely reported but further studies are needed[2]. Aim of this study is to assimilate existing data on medicinal uses and to evaluate the anthelmintic properties against larvae of *Haemonchus contortus* and *Toxocara canis* through *in vitro* studies.

Material and Method

This study consisted of two components, that is; literal study on medicinal uses of *V. cinerea* and *in vitro* studies on anthelmintic properties against larvae of *Toxocara canis* and *Haemonchus contortus.*

(a) Literal study on medicinal uses of *Vernonia cinereal* Medicinal uses of *Vernonia cinerea* (*Monerakudumbiya*) were gathered from Ayurveda and Sri Lankan traditional medical books, interviewing physicians, research journals and internet.

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(b) *In vitro* studies on Anthelmintic properties against larvae of *Toxocara canis* and *Haemonchus contortus*

Preparation of decoction of *Vernonia cinerea* (*Monerakudumbiya*)

Decoctions were prepared according to Ayurveda norms and rules on preparation of drugs (Ayurveda *Paribhasha*). According to Ayurveda *Paribhasha*, 120 g (24 *Kalan*) of fresh materials are mixed with 1920 ml (8 *Patha*) of water and boiled down to one eighth of it, that is; 240 ml (1 *Patha*) to prepare decoctions.

Anthelmintic properties against larvae of *Toxocara canis*

Collection of *Toxocara canis* eggs

Eggs of *Toxocara canis* were obtained from the faeces of young puppies and were embryonated for larval preparations as described by Rajapakse *et al*., (1992)[3]. Before collection of samples from the puppies, faeces were screened by direct smear method. The faecal samples were collected from all the positive animals. All positive samples were mixed in one litre of water containing 0.05% Teepol (Lankem Ceylon Ltd., Colombo) in a measuring cylinder and washed five times by sedimentation method to remove all fat and other fine materials.

Thereafter, the sediment was re-suspended in 500ml of saturated salt solution and the suspension was centrifuged (at 1000g for 10 minutes) in order to separate the eggs. The surface layer of the supernatant solution containing the *T. canis* eggs was collected using a Pasture pipette and washed with water through a filter of 100µm pore size in order to remove the coarse fibrous matter. The filtrate was then poured through a filter of pore size 50µm where *T. canis* eggs remained on the filter.

Development of infective eggs of *Toxocara canis*

Freshly harvested eggs were stored in 0.1 N H_2SO_4 at a depth of 0.5 cm in Petri dishes (10 cm x 1.5 cm) in an incubator (Lindberg and May Pvt. Ltd., Australia) at 14.5 ^{0}C. At this temperature the development of eggs was arrested without any substantial reduction of their viability. The eggs could be stored in this manner for 60 days. Whenever infective eggs were required, Petri dishes containing the required number of eggs were placed at room temperature (22^{0}C - 24^{0}C). In the course of this second incubation, the culture was rocked gently once a day to ensure aeration. Eggs reach infective stage within 30-40 days. Thereafter eggs were washed twice by centrifugation at 150g for 15 minutes with 0.15 M Phosphate Buffered saline (PBS) (pH 7.2) to remove H_2SO_4 and the other organic matter and the eggs were recounted at 1:100 dilutions by the McMaster technique. Viability of the *T. canis* embryonated eggs was assessed by the light stimulation method before use as described by O'Lorcain *et al*. (1995).

The storage and maintenance of larval cultures

The storage and maintenance of larval cultures of *Toxocara* larvae to be used for experimental purposes often have to be stored, and this was done satisfactorily in a shallow layer of water. Forty millilitres of a suspension containing not more than 3000 larvae per ml were placed in a tissue culture flask and kept in an incubator maintained at 10 ^{0}C. As the storage at low temperatures would induce inhibition of some population, care was taken not to use larvae while they were being conditioned. This means that the usage before 4 weeks of storage (larvae had been stored for 2 weeks to ensure a normal establishment rate) or after 16 weeks, was avoided.

***In vitro* larval migration inhibition assay**

The larvae migration inhibition (LMI) bioassay developed by Wagland *et al*. (1992) and modified by Rabel *et al*. (1994)[4] was used to determine the effectiveness of the twenty five plant extracts against infective larvae.

Decoction of *Vernonia cinerea* (*Monerakudumbiya*) was diluted by adding Phosphate Buffered Saline (PBS. One millilitre of the solution was taken and diluted with 29 ml of PBS so as to obtain a transparent solution. Then the density was measured in these solutions. As the positive control levamisole 200 µg/ml was used, whereas phosphate buffered saline (PBS) was used as the negative control.

Then 200 µl of larval suspensions were added to wells containing 800 µl of either controls (positive and negative) or plant extract and were incubated at 37^o C in the wells of tissue culture plates. Three wells (replicate samples) were run for each concentration of each decoction and for the controls.

All the incubations were carried out in 24 well tissue culture plates overnight (18 hours), at 37 ^{0}C and pH 7.2. Following day solutions were transferred to sieves (20 μm mesh at one end) and left for 24 hours at room temperature for active larvae to migrate through the sieves, which were counted later.
On the next day, sieves were removed, Lugol's iodine (0.1 ml) was added to the well and the number of larvae which had migrated was counted under the microscope. The viability and activity of the post migratory larvae with different plant remedies were observed and recorded as follows.

Grade 0 = Dead; No recovery after prolonged immersion in saline
Grade 1 = Inactive but occasional movement can be observed;
Grade 2 = Inactive but intermittent movement can be observed clearly;
Grade 3 = Slow moving;
Grade 4 = Active.

Anthelmintic properties against larvae of *Haemonchus contortus* (*in vitro*)

Collection of the eggs of *Haemonchus contortus*
Fecal samples were collected directly from the rectum of goats in Kekirawa veterinary range, Sri Lanka. Fecal egg count was determined using the modified McMaster technique (Cringoli, 2011). Faeces of high eggs per gram (EPG) of >5000 from each animal were collected for this study. All positive samples were then subjected to fecal culture for collection of infective larvae.

Fecal culture and isolation of *Haemonchus contortus* larvae
Faecal cultures were prepared using faeces collected from infected goats. The faeces were broken up finely, using a large pestle and mortar, mixed with sterile dung or sawdust in 1:1 ratio, and dampened with distilled water until the mixture was moist and crumbly. Then the mixture was kept in wide-mouthed glass jars or enamel trays and incubated at room temperature for 10-14 days. The cultures were maintained by aerating the lower layers every day and, to prevent drying, by adding a few drops of water in order to maintain moisture. After 14 days, cultures were baermannized using wide-mouthed glass jars. The larvae were counted and assessed for viability and identification was carried out to the level of genus before being stored at 10 ^{0}C.

The storage and maintenance of larval cultures
The storage and maintenance of larval cultures were carried as described under *Toxocara canis.*

***In vitro* larval migration inhibition assay**
In vitro larval migration inhibition assay was carried out as described under *Toxocara canis* but infective larvae in unsheathed forms were used**.** The *Heamonchus* infective larvae that were subjected to test were in unsheathed forms. Sheathes were removed by incubating the larvae in sodium hypochlorite solution (0.025% available chlorine) for 10 minutes at room temperature, washing several times and concentrating to approximately 2500 larvae/ml PBS.

Results
Review on Vernonia *cinerea* (*Monerakudumbiya*)
Scientific classification **of** *Vernonia cinerea* (*Monerakudumbiya*)

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Asteridae
Order:	Asterales
Family:	Compositae
Genus:	Vernonia
Species:	cinerea

Synonyms of *Vernonia cinerea* (*Monerakudumbiya*)

Sinhala name:	*Monerakudumbiya*
Sanskrit name:	*Sahadevi, Uttamkanyaka, Dandotpalaa.*
English name:	Purple Fleabane, Ash coloured Fleabane
Tamil name:	*Naichotte Poonde, Seedeviyar shenkaluneer*
Botanical name:	*Vernonia cinerea, Cyanthillium cinereum* (L.) H. Rob.

Description of *Vernonia cinerea* (*Monerakudumbiya*)

An annual herb with slightly branched, stiff, erect, cylindrical, more or less pubescent stem 15-60 cm tall; Leaves simple, alternate, distant, the lower ones 4-5 cm long, 3-3.5 cm broad, gradually becoming smaller upwards. Broadly oval to linear-lanceolate, tapering to the base, sub-obtuse, apiculate, coarsely and shallowly crenate-serrate, more or less hairy on both sides, Petioles 0.6-1.8 cm long; Flowers regular, bisexual, pinkish violet, all tubular, sessile on long, stalked, small heads in divaricate, terminal corymbs, involucre-bracts linear to oblong. 1.5-2.5 mm long, muctonate, silky outside, flowers 20-25 in a head; sepals reduced into long bristles with a shorter outer row; petals 5, fused into a long, tubular corolla about 4 mm long, segments deep and narrow; stamens 5, on the corolla tube, anther not tailed at the base; ovary 1mm long; hairy, inferior, 2-carpellary, unilocular with a single basal ovule, style stout, 3.5 mm long, stigma bilobed ; fruit a hairy achene, 1.5-2 mm long but not ribbed, with a white pappus the outer row of which is short [5] (Figure 1 and 2).

Figure 1: Plant of *Vernonia cinerea*

Figure 2: Flowers of *Vernonia cinerea*

Distribution of *Vernonia cinerea* (*Monerakudumbiya*)

Occurs throughout Sri Lanka, India, tropical Asia, Africa and Australia (Figure 3). In Sri Lanka, it is a very common weed everywhere[5]. It can be seen in roadside, open waste places, dry grassy sites and in perennial crops during plantation.

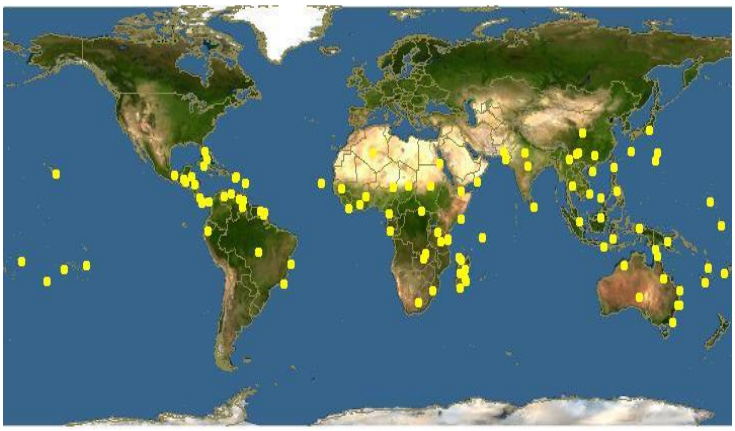

Figure 3: Distribution of *Vernonia cinerea*

Ayurveda pharmacodyanamic properties of *Vernonia cinerea* (*Monerakudumbiya*)[6]

Rasa: *Tikta*
Guna: *Laghu, Ruksha*
Veerya: *Ushna*
Vipaka: *Katu*
Dosha Karma: Kapha Vata Shamaka

Part use in Medicine: Leaves, flowers, seeds, root, entire plant

Medicinal uses of *Vernonia cinerea* (*Monerakudumbiya*)

Vernonia cinerea (*Monerakudumbiya*) has been used externally and internally to treat a number of disorders, it is used singly or in combination with various medicaments. Some selected formulae prepared with *V. cinerea* are given below.

i. Juice is extracted from fresh leaves and 10-20 ml is given to treat dysentery, colic and piles[5].
ii. Decoction is prepared with entire fresh plant and 120 ml of the decoction is given to treat cough, bronchitis and bronchial asthma[5]
iii. A paste prepared with leaves is applied to reduce pain and swelling (*Shota*)[6].
iv. Paste prepared from leaves is applied to the eye in conjunctivitis[6].
v. Paste prepared from leaves is applied in disorders in nervous system[6].
vi. The crushed leaves are applied externally on wounds and sores[5].
vii. In fever, a root is tied on the head and juice is applied on the body[6].
viii. Chronic fever and periodically recurrent fever are managed with decoction prepared with entire plant of *Vernonia cinerea*[6].
ix. A paste prepared with leaves is applied for Ringworm[7]
x. 120ml of decoction prepared from entire plant is given twice a day in treatment of diarrhoea, stomach-ache and intestinal colic[8].
xi. To treat urinary incontinence in children,10-20 ml of fresh juice of entire plant is given [8].
xii. In Dysuria and renal calculi, 120 ml of decoction of entire plant is given [8].
xiii. 120ml of decoction of entire plant is given twice a day in treatment of psoriasis, vitiligo and other ailments in skin [8].
xiv. Worm infections (round worm and thread worm) are treated by giving 120 ml of decoction prepared with fresh entire plant [8].
xv. Seeds are ground into a paste, mixed with lime juice and applied to destroy Pediculi [8].
xvi. In snake bites, 4 gm of roots of *V. cinerea* is boiled in water, strained through a piece of cloth and taken orally 3-4 times a day[9].
xvii. In gynecological disorders, *V. cinerea* is used orally to treat leucorrhoea and control excessive menstruation[10]
xviii. Roots and leaves of *V. cinerea* are chewed raw or entire plant is boiled in water and drunk to cure sexual impotency and erectile dysfunction[11].
xix. Herbal gruel prepared with entire plant of *Vernonia cinerea* is administered in jaundice and hepatitis, as a home remedy in Sri Lanka.
xx. Fifteen grams each of entire plant of *Vernonia cinerea* and *Phyllanthus debilis, stem of Tinospora cordifolia* and dried fruits of *Phyllanthus emblica* are added to 1920 ml of water and boiled down to 240ml. 120ml of this decoction is given twice a day to treat epistaxis[12].

Veterinary uses

Seeds are given to the animals to treat food poisoning. Infusion of seeds is given to the livestock animals to cure fever[13].

Bark of babul, seeds of *Trachyspermum ammi* and *V. cinerea* mixed with jaggery is given to the livestock

Bark of babul, seeds of *Trachyspermum ammi* and *V. cinerea* mixed with jaggery is given to the livestock wise a day for one month as a tonic to cure overall weakness[13].

Some animals such as wild chimpanzees are believed to ingest *Vernonia cinerea* when suffering from cancer.

Chemical constituents

Preliminary phytochemical screening revealed the presence of flavonoids, glycosides, tannins, and carbohydrates in *Vernonia cinerea.*[14] It also contains flavonoids, saponins, alkaloids, and terpenoids.[15]*V. cinerea* contains vernolide-A and vernolide-B (two novel sesquiterpene lactones); β-amyrin, lupeol and their acetates; and β-sitosterol, stigmasterol, α-sp inasterol and phenolic resin in the whole plant. The roots contain δ-amyrin acetate, α-amyrin acetate, β-amyrin acetate, β-amyrin and α-amyrin. In addition, the leaves contain urticifolene (new polyene), lutein (carotenoid) and sitosterol (triterpenoid). The stem, bark and leaves contain lupeol, 12-oleanen-3-ol-3β-acetate and stigmasterol.[16]

Research

i. Reddy *et al*., (2012) evaluated the anticataleptic efficacy of ethanol extract of *Vernonia cinerea* L. in haloperidol induced catalepsy in rats. Scientific evaluation of this claim using experimental model Anticataleptic activity using block method, Locomotor activity in actophotometer and

Exploratory behavior in hole board apparatus. From the observations of above studies, it could be envisaged, that the protective effect of ethanol extract of *Vernonia cinerea* L. against symptoms of Parkinson's disease (catalepsy) may be due to regulation in neurotransmitters such as dopamine, serotonin, glutamate which are playing an important role in protection of catalepsy and antioxidant properties[17].

ii. Ganesh *et al*., (2011) further confirmed the antidiarrhoeal activity of methanolic extract of *Vernonia cinerea* L., (Less) and reported dose dependent[18].For evaluation of antidiarrhoeal efficacy of methanolic extract of the plant, rats were used as test animal. The time of onset of first wet faeces increased significantly and dose dependently by the extract. It was excellent at higher doses (100 and 200 mg/kg body wt., orally).

iii. Ngbolua *et al*., (2011) detected moderate antiplasmodial activities in *V. cinerea* subsp vialis; a plant species not previously reported as antimalarial in the traditional medicinal knowledge of Madagascar[19].

iv. Latha *et al*., (1998), tested anti-inflammatory effect of an alcoholic extract from the flower of *Vernonia cinerea* (Asteraceae) in adjuvant arthritic rats. Changes in paw volume, body and tissue weights and, serum and tissue enzyme activities of ALT, AST, ACP and cathepsin-D in adjuvant rats were reversed by oral administration of 100 mg of the flower extract per kg of body weight (BW). The extract also reversed the major histopathological changes in the hind paws of the arthritic rats[20].

v. Bashar *et al*., (2014) reported antipyretic, analgesic and anti-inflammatory activities of the methanol extract of whole plant of *V. cinerea* Less. Antipyretic activity was assessed by the yeast-induced hyperthermia in mice. The analgesic property was evaluated by formalin-induced writhing test. Acetyl salicylic acid (ASA) was used as standard in in-vitro anti-inflammatory activity test [21].

vi. Ushasri *et al*., (2013) evaluated the alcoholic, etheral and chloroform extracts were obtained from the roots of plant *Vernonia cinerea* by soxhlet extraction or continuous hot percolation methods for their respective anthelmintic activity, against locally available earth worms (*Pheretima posthuma*). Three concentrations (10, 30, 60 mg/ml) were prepared from each extract and used for the study over earth worms. The study involves the determination of time of paralysis and time for death of the earth worms tested. The results obtained from the study revealed the fact that chloroform and alcoholic extracts from the roots of *Vernonia cinerea* possess significant anthelmintic effect[22].

vii. Toyang and Verpoorte (2013) found that *Vernonia cinerea* has potential against cancer and inflammatory conditions according to reported literature. Vernolide A is so far the most promising single agent from a *Vernonia* species that has potential for development into an anticancer agent[23].

viii. Dakshini *et al*., (1992) found that the concentrations of heavy metals i.e. cobalt, copper, nickel, manganese and zinc in dried material of *Vernonia cinerea* are much higher than in the soil samples. Accumulation of these metals is greater in pink than in purple or mauve flowered forms[24].

ix. Asha and Abraham (2015) had evaluated the efficacy of methanolic extract of *Vernonia cinerea* (MEVC) in selenite induced cataract using Sprague Dawley rats. MEVC was administered as orally from 8th day up to 21st day at the concentration 5 µg/g body weight. The findings, suggest that *V. cinerea* has the therapeutic potential of lens against selenite induced cataract. It is possible that *V. cinerea* might be useful against lens damage caused by ROS generation under oxidative stress. It is also relatively nontoxic when given in small doses. Hence, these findings are considered pharmacologically significant; evaluation of active component from *V.cinerea* will certainly uncover novel therapeutic possibilities[25].

x. Muir (1981) reported that the aqueous extract of *Vernonia cinerea* contains a depressant agent whose primary effect is that of analgesia. In Malaysia, *Vernonia cinerea* is included in several traditional herbal preparations used for insomnia and related ailments. Most preparations of *Vernonia cinerea* are concoctions where the plant is boiled in water. Any active ingredient is therefore presumably water soluble and heat stable. Though these results are preliminary in nature, they may suggest that the use of aqueous extracts of *Vernonia cinerea* for its sedative effect may entail hitherto unknown dangers since effective sedative actions in mice appear to occur only at relatively high doses but that the plant may contain an agent which might be of use at relatively lower (and therefore safer) doses for the control of pain[26].

xi. Dhanalakshmi *et al*., (2013) reported that ethyl acetate extract of *Vernonia cinerea* exhibited excellent antidandruff activity against Pityrosporum ovale and Pityrosporum folliculitis. The antifungal activity of *V. cinerea* leaf extracts showed positive results against all the tested fungal pathogens; *C. albicans, C. parapsilosis* and *C. tropicalis*[27].

xii. Leelarungrayub *et al*., (2010) carried out a study to evaluate the effects of *Vernonia cinerea* Less. (VC) supplementation and exercise on oxidative stress biomarkers, beta-endorphin release, and the rate of cigarette smoking. 20gm of dried entire plant of *V. cinerea* mixed with 390ml of water and boiled in an earthen pot until water evaporated down to 130ml. Condensed VC juice was then preserved in a clean bottle and was provided to subjects to drink prior to smoking each, three days per week for two months. Supplementation with *V. cinerea* Less and exercise provided benefit related to reduced smoking rate, which may be related to oxidaive stress and beta-endorphine levels[28].

xiii. Sreedevi (2011) studied nephroprotective activity of *V. cinerea*. The alcoholic extract of *Vernonia cinerea* showed promising nephrocurative activity, whereas ethyl acetate extract of *Vernonia cinerea* possessed significant nephroprotective activity in the rat model of cisplatin induced renal toxicity. These results suggest the therapeutic utility of herbal *Vernonia cinerea* extracts in renal injury[29].

xiv. An *in vivo* study showed that *V. cinerea* had an equivalent antipyretic effect to paracetamol when the extract was taken at a dose of 500mg/kg in rats (Gupta, 2003)[30].

Physio-chemical composition of *Vernonia cinereal (Monerakudumbiya)*

Madanayaka *et al.,* (2015) studied physio-chemical composition of root of *Vernonia cinerea* in 100gm. It contains 68.8 gm moisture. Ash value is 2.81g. The researchers reported that it also contains crude protein (2.36gm), crude fibre (13.73 gm), crude fat (0.81 gm) and water soluble sugars (38.5gm)[31].

Anthelmintic properties against **larvae of *Toxocara canis* and *Haemonchus contortus* (*in vitro*)**

As shown in the table 1, decoction of *V. cinerea* was 89.4 % effective in inhibiting *Toxocara* larval migration and 86.7% effective in inhibiting *Haemonchus* larval migration. Whereas larval migration inhibition of *Toxocara canis* and *Haemonchus contortus* with Levamisole were 99.7% and 96.6% respectively.

The viability of post-migratory larvae of *Toxocara canis* and *Haemonchus contortus* with controls and with decoction of *V. cinerea* are presented in Table 2. Maximum number of migrated larvae of *Toxocara and Haemonchus* was observed in the negative control PBS. Least number of migrated larvae was observed in the positive control Levamisole and all the larvae were dead after migration. 6.7% of *Toxocara* and 4.4% of *Haemonchus* larvae were migrated in decoction of *V. cinerea*. All the migrated *Toxocara* larvae were dead or in Grade 1, 2, 3 or 4 and in *Haemonchus* larvae were dead or in Grade 1.

Table 1: Percentages of *in vitro* larval migratory inhibition of *Toxocara canis* and *Haemonchus contortus* infective larvae with decoction of *Vernonia cinerea*

Treatment	Percentage (%) of larval migration inhibition (LMI)	
	Toxocara canis	***Haemonchus contortus***
Levamisole 200 µg / ml in PBS (Positive Conrol)	99.7	96.6
Phosphate buffered saline (Negative control)	0	0
Vernonia cinerea (*Monerakudumbiya*)	89.4	86.7

Table 2: Viability of post-migratory larvae of *Toxocara canis* and *Haemonchus contortus* larvae with decoction of *Vernonia cinereal*

Treatment	Percentage (%) of viability of *Toxocara canis* larvae						Percentage (%) of viability of *Haemonchus contortus* larvae					
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Total**	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Total**
Levamisole (Positive control)	55	0	0	0	0	55	0.1	0	0	0	0	0.1
Phosphate buffered saline (Negative control)	0	0	0	0.2	61.5	61.7	0	0	0	0	72.5	72.5
Vernonia cinerea (*Monerakudumbiya*)	0.2	0.1	0.9	1.3	4.2	6.7	2.2	2.2	0	0	0	4.4

Grade 0 = Dead; No recovery after prolonged immersion in saline; Grade 1 = Inactive but occasional movement can be observed; Grade 2 = Inactive but intermittent movement can be observed clearly; Grade 3 = Slow moving; Grade 4 = Active

Discussion

Vernonia cinerea (*Monerakudumbiya*) is used to treat oedema, fever, jaundice, skin diseases, epistaxis, urinary incontinence, dysuria, renal calculi and Parkinsonism. Antipyretic, anti-inflammatory, analgesic, antidiarrhoeal, anti-cancer, antitumor. antidandruff, antiplasmodial, anticataleptic, nephroprotective activities and anthelmintic activity against earth worms (*Pheretima posthuma*) are scientifically proven.

Decoction of *V. cinerea* was 89.4 % effective in inhibiting *Toxocara* larval migration and 86.7% effective in inhibiting *Haemonchus* larval migration. According to Ayurveda *V. cinerea* possesses *Tikta Rasa, Laghu and Ruksha Guna, Ushna Veerya* and *Katu Vipaka.* These properties lead to reduction of *Kapha Dosha*. Ayurveda describes three methods to treat *Krimi Roga* (worm infection)[8]. One of them is *Prakruti Vighata*, that is making the environment unfriendly for worms. *V. cinerea* makes the surrounding unfriendly to worms by reducing *Kapha Dosha* in the environment. Further, Ayurveda describes *Krimighna* (wormicide) property of *V. cinerea* which leads to killing of the worms. Therefore, *V. cinerea* is effective in inhibiting *Toxocara* larval migration and *Haemonchus* larval migration. Iqbal et al., (2007) reported that tannins has anthelmintic effect[32]. β-sitosterol possess *in vitro* anthelmintic properties against sheep GIS.[33] Alkaloids, Phenols and tannins are accountable for anthelmintic activity.[34] As *Vernonia cinerea* contain these phytochemicals, it possesses anthelmintic properties and it inhibits *Toxocara* larval migration and *Haemonchus* larval migration.

Conclusion

It is concluded that *Vernonia cinerea* (*Monerakudumbiya*) is effective in inhibiting larval migration of *Toxocara canis* and *Haemonchus contortus* and also has multi-faceted medicinal values.

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Comparative analysis of phytochemical and antioxidant activities of the *Nishatipal* decoction and it's freeze dried powder

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Abstract

Nishatipal decoction is a traditional polyherbal formulation which is used extensively by Sri Lankan Ayurvedic and Traditional medical practitioners to treat Diabetes Mellitus. Eight different medicinal plants are present in this decoction. The aim of the present study was to establish phytochemical, chromatographic analysis of the decoction and its freeze-dried powder. In addition, *in-vitro* antioxidant activity was evaluated for the freeze-dried powder of *Nishatipal* decoction. Phytochemical screening of the decoction and its freeze-dried powder exhibited many primary and secondary metabolites. Organoleptic properties indicated, the decoction was a dark brown liquid with a characteristic odor, bitter taste and sticky on touch. pH and density of the decoction were 5.22 and 1.009075g/cm^3 at 27ºC. Furthermore, peaks at similar R_f values for both decoction and freeze-dried powder indicated the presence of similar bioactive compounds. *In-vitro* antioxidant activity revealed that total polyphenolic content and DPPH radical scavenging activity were in significant levels in freeze dried form of *Nishatipal* decoction. The findings from this study provided evidence that the decoction contains medicinally important bioactive compounds which help amelioration of Diabetes Mellitus. In addition, quality control parameters were established for *Nishatipal* decoction and its freeze-dried form for the first time.

Keywords: Antioxidants, *Nishatipal*, Phytochemicals, TLC, HPTLC,

Introduction

Natural products, specially the plant extracts have been an inspiration for the production of novel drugs. The use of herbal medicine is becoming more popular in the world day by day, due to the increment in the trend of people returning towards the use of herbal therapeutics for their ailments[1]. A huge scientific literature has been focusing on bioactivities of Sri Lankan medicinal extracts over the past recent years. However, chemical standardization was carried out for a tiny minority of plant species[2]. One of the impediments in the acceptance of the herbal extracts among general public is the lack of chemical standardization, therefore the standardization of the herbal drugs in accordance with acceptable guidelines have become utmost essential[2,3,4].

Nishatipal decoction is a traditional polyherbal formula specially indicated for the treatment of diabetes in authentic traditional medicine books in SriLanka[5]. *Nishatipal* decoction consists of *Curcuma longa* L., *Terminalia chebula* Retz., *Terminalia bellirica* Roxb., *Phyllanthu semblica* L., *Coscinium fenestratum* Colebr., *Cyperus rotundus* L., *Strychnos potatorum* L.f. and *Terminalia arjuna* Roxb.[5].

In Ayurveda and traditional medicine "Diabetes" comes under the disease "*Prameha*". Diabetes mellitus (DM) is a fast growing non communicable disease around the world, particularly in the developing countries. In South Asia, DM has been identified as a growing and a major contributor towards the mortality and disability. Its prevalence has risen from 4.7% in 1980 to 8.5% in 2014[6].

The aim of the present study was to establish quality control parameters of the *Nishatipal* decoction (NTD) and its freeze-dried powder. In addition, freeze dried powder of *Nishatipal* decoction was subjected for *in vitro* antioxidant activity.

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Materials and Methods

Selection, Authentication and Processing of the raw materials

All the dried raw materials (Table 1) were washed well to remove adhered foreign matter, dried in the shade and stored in separate air tight bottles. The plants used in NTD were collected from Colombo city (6° 55' 54.98'' N x 79° 50' 52.01'' E) Western province, Sri Lanka, between July and August 2018 and authenticated by the Curator at the Department of *Dravyaguna Vignana*, Institute of Indigenous Medicine, and University of Colombo, Sri Lanka.

Table 1: **List of raw materials in *Nishatipal* decoction**

Botanical name of the plant	Used part of the plant	Proportions (weight basis)
Curcuma longa L.	Rhizome	1
Terminalia chebula Retz.	Fruit	1
Terminalia belerica Roxb.	Fruit	1
Phyllanthus emblica L.	Fruit	1
Coscinum fenestratum Colebr.	Stem	1
Cyprus rotundus L.	Tubers	1
Strychnos potatorium L.f.	Seeds	1
Terminalia arjuna Roxb.	Bark of the stem	1

Preparation of *Nishatipal* powder

Each of the dried raw materials was ground to a fine powder using a blender and accurately weighed 7.5g of each of it and mixed well together to make a powder mix of *Nishatipal*.

The following standardization tests were done to the powder mix of *Nishatipal.*

Determination of total ash, water soluble ash and acid insoluble ash in *Nishatipal* Powder Mix

Percentage of total ash, water soluble ash and acid insoluble ash contents were determined according to methods described in WHO standards[7].

Preparation of *Nishatipal* decoction

The coarse powder mix of *Nishatipal* was kept in a large clay pot and 1920.0 mL of water was added[8].The mixture was heated under moderate fire for 4 to 5 hours until the total volume decreased to 240.0 mL. Decoction was allowed to cool and filtered through a cheese cloth, cotton wool bed and then through a filter paper. The filtrate was used to perform phyto-chemical tests.

Quality Control and Standardization of *Nishatipal* decoction

Organoleptic Properties

The decoction was inspected for its colour, odour, appearance, texture and touch.

Determination of PH

The pH of the filtrate was determined[7] using the pH meter (pH 700, Singapore).

Determination of Density

The density of the filtrate was measured using the intelligent density meter (BHDM 2l 2003201110612, Japan).

Qualitative Phytochemical Analysis

Tests for Primary Metabolites

The tests for primary metabolites were carried out by the methods described in[9]with some modifications (Table 2).

Tests for Secondary Metabolites

The tests for secondary metabolites were carried out by the methods described in[10] with some modifications (Table 3).

Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) fingerprints of *Nishatipal* decoction

Filtered decoction (100.0 mL) was taken into a 1.0 L separating funnel and 100.0 mL of distilled water was added to it for dilution. Dichloromethane (50.0mL) was added to the separating funnel and shaken vigorously for efficient transfer of compounds from aqueous layer to the organic layer. The mixture was allowed for phase separation for 30 minutes. The bottom dichloromethane layer was collected carefully to a well cleaned and dried reagent bottle. The aqueous layer remaining in the separating funnel was extracted for two times using two more 50ml portions of dichloromethane using the same procedure. The organic layers in the latter two extractions were also collected to the same reagent bottle, was completely dried using rotavapour and dissolved in 5.0mL of dichloromethane. This solution was used for TLC spotting.

Table 2: **Tests for primary metabolites in *Nishatipal* decoction**

Primary Metabolites	Test Procedure	Observations
Proteins	**Ninhydrin test:** Filtrate (2 mL) was boiled with 2 mL of 0.2% solution of Ninhydrin.	Violet colour indicates the presence of proteins.
Carbohydrates	**Benedict's test:** Filtrate (2 mL) was mixed with 2 mL of Benedict's reagent and boiled.	Formation of a reddish brown precipitate indicates the presence of reducing sugars.
	Iodine test: Filtrate (2 mL) was mixed with 2 mL of Iodine solution.	Development of a dark blue or purple color indicates the presence of starch.

Table 3: **Tests for secondary metabolites in *Nishatipal* decoction**

Secondary Metabolites	Test Procedure	Observations
Phenolics	**Folin Reagent Test:** To 2 mL of the filtrate, few drops of Folin Reagent were added.	Blue color indicates the presence of phenolics.
	Ferric Chloride Test: To 2 mL of the filtrate, few drops of the $FeCl_3$ were added.	Blue color indicates the presence of phenolics.
	Lead Acetate Test: To 2 mL of the filtrate of the decoction, few drops of Lead Acetate were added.	Yellow color precipitate indicates the presence of flavonols and flavones.
Saponins	**Frothing Test:** To 2 mL of the filtrate, 2 mL of distilled water was added and shaken vigorously.	Persistent froth for at least 10 minutes indicates the presence of saponins.
Flavonoids	To 2 mL of the filtrate, few zinc granules and few drops of concentrated HCl were added and then heated in a boiling water bath.	Development of an orange colour indicates the presence of flavonoids.
	To 2 mL of the filtrate, few drops of NH_4Cl were added.	Development of a yellow color which disappears upon addition of diluted HCl indicates the presence of flavonoids.
Alkaloids	**Dragendorff Test:** To 3 mL of the filtrate, 3mL of 1% HCl was added and heated gently. Then the mixture was divided into three 2 mL portions. To the first 2 mL portion 1 mL of Dragendorff reagent was added.	Development of an orange/ red precipitate indicates the presence of alkaloids.
	Wagner's Test: To the second 2 mL portion, 1 mL of the Wagner's reagent was added.	Development of an orange color precipitate indicates the presence of alkaloids.

	Picric acid Test: To 2 mL of the filtrate, few drops of picric acid were added.	Development of a yellow crystalline precipitate indicates the presence of alkaloids.
	Tannic acid Test: To 2 mL of the filtrate, few drops of tannic acid were added.	Formation of a yellow crystalline precipitate indicates the presence of alkaloids.
Tannins	**Vanillin Test:** To the 2 mL of the filtrate, few drops of 10% vanillin in ethyl alcohol were added.	Development of red color indicates the presence of tannins.
	Lead Acetate Test: To 2 mL of the filtrate, few drops of lead acetate were added.	Formation of a yellow color precipitate indicates the presence of tannins
Steroids	**Lieberman Burchard Test:** To 3 mL of the filtrate, 2 mL of chloroform was added. Then 0.5 mL of acetic anhydride and 0.5 mL of concentrated H_2SO_4 were added.	Development of blue or green color indicates the presence of steroids.
Terpenoids	**Salkowski Test:** To 2 mL of the filtrate, 2 mL of chloroform was added and mixed well. Then 3 mL of concentrated H_2SO_4 was added along the sides to form a layer.	Formation of reddish brown color indicates the presence of terpenoids.
	Test for Monoterpenes: To 2 mL of the filtrate, few drops of 10% vanillin in ethanol and few drops of concentrated H_2SO_4 were added.	Development of a red color indicates the presence of monoterpenes.
	Test for Sesquiterpenes: To 2 mL of the filtrate, few drops of concentrated H_2SO_4 were added.	Development of a red/blue color indicates the presence of sesquiterpenes.

Development of Thin Layer Chromatography (TLC) Fingerprint

The dichloromethane extract of the decoction thus prepared was spotted on a TLC plate (precoated sheets ALUGRAM Xtra SIL, 8cm × 10cm, 0.20mm thickness). The plate was developed using hexane, dichloromethane and methanol in a ratio of 1:8.5:0.5. The developed plate was dried in air and viewed for spots using a UV lamp (CAMAG TLC scanner, Switzerland).

Development of High Performance Thin Layer Chromatography (HPTLC) fingerprint

HPTLC screening of the developed TLC plate was done using the CAMAG HPTLC scanner (Switzerland).

preparation of the freeze dried powder from the *Nishatipa*l decoction

Nishatipal decoction (240ml) was allowed to cool and filtered through a cheese cloth, cotton wool bed and then through a filter paper respectively. This filtrate was freeze dried at -52℃ using the freeze dryer, LABCONCO (U.S.A) until a fine powder was obtained. This was stored in a sterilized air tight plastic container in a freezer for future purposes.

Analysis of the freeze dried powder of *Nishatipal* decoction

Organoleptic properties

Freeze dried powder of *Nishatipal* decoction was inspected for its colour, odor, texture, appearance and touch.

***Invitro* analysis of Antioxidant activity**

Determination of Total Phenolic Content

This was done by Folin Ciocalteu's method as described by Singleton and co-worker[11].

Preparation of the sample

Freeze dried powder of the decoction (2 mg) was dissolved in 40 µl of DMSO (dimethyl sulfoxide) and then it was diluted by adding 960 µl of distilled water to give a concentration of 2 mg/ml.

Preparation of the standard

Gallic acid (1mg) was dissolved in 1ml of distilled water to give up a solution with concentration of 1mg/ml to use as a stock standard.

Preparation of the sample series

The sample series was prepared by diluting the stock solution of the sample with distilled water to get four concentrations (2 – 0.25 mg/ml).

Preparation of the standard series

The standard series was prepared by diluting the stock standard with distilled water to get eight concentrations ($1 – 7.81\times10^{-3}$ mg/ml).

Recording the absorbance

In a micro plate, 110µL of Folin Ciocaltaeu reagent was mixed with 20µl of each standard/sample in four replicates. A pre plate reading was taken at 765nm using a UV visible spectrophotometer. Then 70 µL of 10% sodium carbonate solution was added to each. The mixtures were incubated at room temperature for 30 minutes. The absorbance values were read at 765nm. Distilled water was used as the blank. Gallic acid was used as the reference standard and the gallic acid equivalent for the samples were calculated. The result of the total phenolic content was expressed as milligrams of gallic acid equivalents per gram of the freeze-dried powder.

Analysis of 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity

This was done by the method described as Blois[12]with some modifications.

Preparation of the sample

Freeze dried sample (2.0mg) was dissolved in 100.0µL of DMSO (dimethyl sulfoxide) and 900µl of distilled water to get a stock sample with a concentration of 2 mg/ml.

Preparation of the standard

Trolox 1 mg/ml solution was prepared as the standard.

Preparation of the sample series

The sample series was prepared by diluting the stock sample solution with methanol to give eight concentrations ($2 – 1.56\times10^{-2}$ mg/mL).

Preparation of the standard series

The standard series was prepared by diluting the stock standard with methanol to give eight concentrations ($1 – 7.81\times10^{-3}$ mg/mL).

Recording the absorbance

In a micro plate, 50 µL of each sample/standard was dissolved with 100µL of methanol in four replicates. A pre plate reading was taken at 517nm. DPPH solution (50µL) was added to each. The mixtures were incubated at room temperature for 10 minutes. The absorbance was read at 517nm. A mixture of 150µL of methanol and 50µlLof DPPH was used as the control and 200µL of methanol was used as the blank. The DPPH radical scavenging activity was calculated as milligrams of trolox equivalents per gram of the freeze-dried powder.

Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) screening of the *Nishatipal* freeze dried powder

Preparation of the extract

Freeze dried powder (approx. 2.0g) was dissolved in 100.0 mL of hot water. It was shaken for 15minutes in a shaker at 160rpm. This solution was then filtered. The filtrate (25.0mL) was taken into a 500.0mL separating funnel and 25.0mL of distilled water was added to it for dilution. It was extracted three times using three 20.0mL portions of the dichloromethane. The organic extract was concentrated and used for TLC spotting.

Development of the fingerprints *Nishatipa*l freeze dried powder

The dichloromethane extract thus prepared was spotted on a TLC plate (precoated sheets ALUGRAM Xtra SIL, 8cm × 10cm, 0.20mm thickness). The plate was developed using hexane, dichloromethane and methanol in a ratio of 1:8.5:0.5. The developed TLC plate was then air dried and the HPTLC screening of the developed plate was done using the CAMAG HPTLC scanner.

Qualitative Phytochemical Screening of the *Nishatipal* freeze dried powder

Preparation of the extract

Freeze dried powder (approx. 6.0g) was dissolved in 100.0mL of hot water. It was filtered through a cotton wool bed and then through a filter paper. The filtrate thus obtained was used for qualitative phytochemical screening.

Tests for primary metabolites

The tests for primary metabolites were carried out by the methods described as[9] with some modifications in the same way as done for the *Nishatipal* decoction (Table 2).

Tests for secondary metabolites

The tests for secondary metabolites were carried out by the methods described as[10] with some modifications in the same way as done for the *Nishatipal* decoction (Table 3).

Results and Discussion

Quality assessment on herbal drugs is very important. Many researchers in Sri Lanka[13,14,15]and also in Asian countries[16,17]have made attempts to establish quality control parameters for herbal drugs. Therefore, in the present study also similar attempt was made to establish the quality control parameters for *Nishatipal* decoction and its freeze-dried form.

A low ash value, acid insoluble ash value and water-soluble ash value (Table 4) were obtained for the raw material powder mix of *Nishatipal*. The quality of the raw materials is essential for the production of herbal products with high quality and required efficacy. Thus, the quality evaluation of raw materials is utmost essential[18].The total amount of the residue after ignition was determined by the total ash method. This included both physiological ash and non-physiological ash. The mineral components of the plant material itself forms the physiological ash whereas, non-physiological ash comes from foreign extraneous matter such as, sand and soil adhered to the plant material by their contact. The residue obtained after the boiling of total ash with diluted hydrochloric acid was the acid insoluble ash. This measures the amount of silica present, mainly as sand and siliceous earth[7]. The water-soluble portion of the total ash was determined as the water-soluble ash value. A very low ash value, acid insoluble ash value and water soluble ash value is indicative of very low contamination and high purity of the raw materials that were used for the preparation of *Nishatipa*l decoction (total ash % : 11.3 ± 0.1, acid insoluble ash %: 0.6 ± 0.1, water soluble ash %: 6.9 ± 0.2). The examination of organoleptic properties of the decoction revealed that it was a dark brown coloured sticky liquid with a characteristic herbal odour. The *Nishatipal* decoction also had very high bitter taste which is highly suitable to be used by individuals with Diabetes and helpful to halt the pathogenesis of diabetes according to Ayurveda[19].

The pH of the freshly prepared decoction was found to be an acidic pH and the density was found to be very close to the density of pure water (pH: 5.22, density: 1.009075g/cm^3).

The qualitative phytochemical screening for the primary metabolites of the *Nishatipal* decoction (Table 04)

Table 4: Results of primary metabolites present in the *Nishatipal* decoction and the freeze-dried powder

Primary metabolite	Test	*Nishatipal* decoction	*Nishatipal* freeze dried powder
Proteins	Ninhydrin test	+	+
Reducing sugars	Benedict's test	+	+
Starch	Iodine test	_	_

(+) indicates presence (-) indicates absence

This result revealed that the decoction contained traces of proteins and reducing sugars, negative results for the Iodine test revealed the absence of starch in the decoction suggesting its suitability to be used by individuals with Diabetes.

The qualitative phytochemical screening for the secondary metabolites of the *Nishatipal* decoction (Table 5). This result revealed that the decoction contained high content of phenolics, saponins, flavonoids and tannins, moderate content of alkaloids, terpenoids, monoterpenes, sequiterpenes and absence of steroids. Phenolics and flavonoids are responsible for the antioxidant and anticarcinogenic properties. They function as free radical scavengers and reducing agents. The saponins are another important group of plant metabolite which has several beneficial properties such as, anti-inflammatory properties, precipitating and coagulating red blood cells, and cholesterol binding properties and also contribute to the bitterness. Alkaloids are one of the most diverse groups of secondary plant metabolites with wide variety of different structures and they are known to produce analgesic, antispasmodic and antibacterial properties[20]. Tannins are known to have high antioxidant and antiglycation properties. The pathogenesis of Diabetic complications such as Diabetic retinopathy, Diabetic nephropathy, Diabetic neuropathy and cardiovascular diseases increases with glycation of proteins and accumulation of end products of advanced glycation. This also accompany with the free radical formation through autooxidation of Glucose. Therefore, the compounds with antiglycation properties have a high potential in reducing Diabetic complications[21]. Terpenoids are another important class of secondary metabolites which are capable of reducing the progression of diabetic complications and act as antidiabetic agents through reducing glucose absorption, increasing insulin secretion, preventing the development of insulin resistance and inhibiting the formation of glycation end products[22]. Steroids can reduce inflammation but it can suppress the immune system and significantly increase the blood glucose levels in individuals suffering with Diabetes, or individuals with impaired glucose tolerance[23]. Therefore, absence of steroids in this *Nishatipal* decoction is highly suggestive of this decoction as an effective treatment for Diabetes mellitus.

TLC is a separation technique used to separate nonvolatile compounds and aid in the separation of a mixture to individual components. The HPTLC is an enhanced form of TLC and it allowed an increased resolution of the separated compounds. The developed TLC plate spotted with the dichloromethane extract of the decoction when viewed under a UV lamp at a wavelength of 366nm (Figure 1).

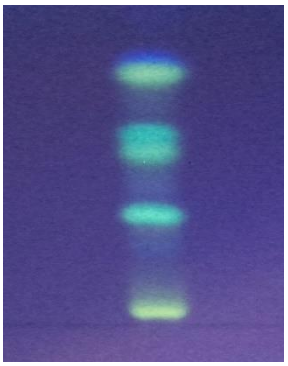

Figure 1: TLC plate spotted with the dichloromethane extract of the *Nishatipal* decoction when viewed through uv lamp at 366nm wavelength

This was showed clearly the separation of spots at different R_f values. The HPTLC screening of the developed TLC plate clearly demonstrated the proportional differences of the R_f values of the separated spots. HPTLC densitogram (Figure 2), and the HPTLC peak table (Figure 3) showed that among them, peaks at R_f values, 0.37, 0.57, and 0.80 were prominent.

A ready to use form of *Nishatipal* decoction was prepared by freeze drying 240.0 mL of the decoction. The preparation of this solid form of the decoction was done to overcome some common difficulties that the people face in using the liquid dosage form of the decoction. The organoleptic properties analysis of the freeze dried powder of *Nishatipal* decoction exhibit brown color, fine and smooth powder with characteristic herbal odour.

Table 5: **Results of secondary metabolites present in the *Nishatipal* decoction and the freeze dried powder**

Secondary metabolite	Test	*Nishatipal* decoction	*Nishatipal* freeze dried powder
Phenolics	Folin reagent test	+++	+++
	Ferric chloride test	+++	+++
	Lead acetate test	+++	+++
Saponins	Frothing test	+++	+++
Flavonoids	Zinc granules + concHCl	+++	+++
	NH_4Cl + dilHCl	+++	+++
Alkaloids	Dragendorff's test	++	++
	Wagners' test	++	++
	Picric acid test	++	++
	Tannic acid test	++	++
Tannins	Vanillin test	+++	+++
	Lead acetate test	+++	+++
Steroids	Lieberman Burchard test	_	-
Terpenoids	Salkwoski test	++	++
	Test for monoterpenes	++	++
	Test for sesquiterpenes	++	++

(+) indicates presence (-) indicates absence

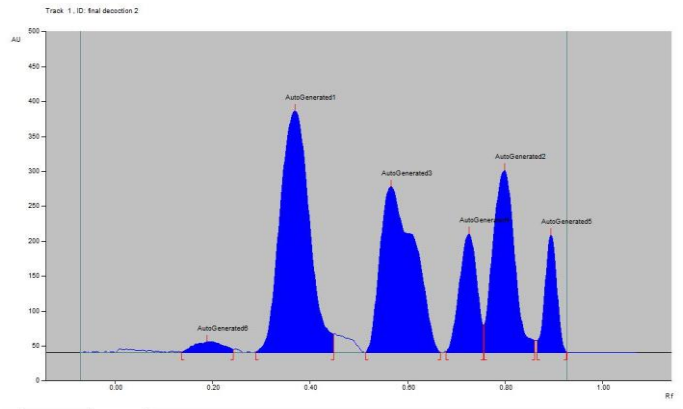

Figure 2: **HPTLC densitogram of the *Nishatipal* decoction**

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.14 Rf	1.1 AU	0.19 Rf	15.9 AU	1.33 %	0.24 Rf	4.4 AU	787.6 AU	1.62 %	AutoGenerated6
1	2	0.29 Rf	1.1 AU	0.37 Rf	346.1 AU	28.86 %	0.45 Rf	27.0 AU	16849.3 AU	34.63 %	AutoGenerated1
1	3	0.51 Rf	0.4 AU	0.57 Rf	237.7 AU	19.82 %	0.67 Rf	0.7 AU	13424.6 AU	27.59 %	AutoGenerated3
1	4	0.68 Rf	2.1 AU	0.73 Rf	170.1 AU	14.19 %	0.76 Rf	38.9 AU	4961.5 AU	10.20 %	AutoGenerated4
1	5	0.76 Rf	40.0 AU	0.80 Rf	261.1 AU	21.77 %	0.86 Rf	17.6 AU	9330.4 AU	19.18 %	AutoGenerated2
1	6	0.87 Rf	17.5 AU	0.90 Rf	168.4 AU	14.04 %	0.93 Rf	1.4 AU	3301.2 AU	6.78 %	AutoGenerated5

Figure 3: HPTLC peak table of the *Nishatipal* decoction

The solid dosage form thus prepared must resemble the efficacy and chemical properties of the original decoction. This was analyzed by performing the HPTLC screening and the qualitative phytochemical screening of the NT freeze dried powder. The HPTLC screening of the developed TLC plate spotted with dichloromethane extract of the freeze-dried powder of *Nishatipal* clearly demonstrated the proportional differences of the R_f values of the separated spots. HPTLC densitogram (Figure 4), and the HPTLC peak table (Figure 5) showed that among them, peaks at R_f values, 0.17, 0.40, 0.61, 0.77 and 0.85 were prominent. The HPTLC screening showed peaks at similar R_f values for *Nishatipal* decoction and its freeze dried powder indicating the presence of similar compounds in both the dosage forms.

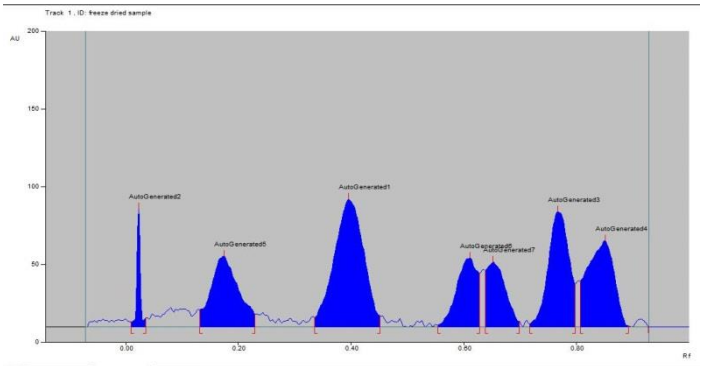

Figure 4: HPTLC densitogram of *Nishatipal* freeze dried powder

Track 1, ID: freeze dried sample

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 Rf	3.2 AU	0.02 Rf	75.8 AU	18.09 %	0.04 Rf	5.8 AU	380.5 AU	3.05 %	AutoGenerated2
2	0.13 Rf	11.0 AU	0.17 Rf	45.6 AU	10.89 %	0.23 Rf	8.2 AU	1798.3 AU	14.40 %	AutoGenerated5
3	0.34 Rf	6.1 AU	0.40 Rf	82.1 AU	19.59 %	0.45 Rf	7.1 AU	3497.0 AU	28.01 %	AutoGenerated1
4	0.55 Rf	1.1 AU	0.61 Rf	44.4 AU	10.59 %	0.63 Rf	34.5 AU	1327.8 AU	10.63 %	AutoGenerated6
5	0.64 Rf	36.1 AU	0.65 Rf	41.7 AU	9.94 %	0.70 Rf	3.5 AU	1150.3 AU	9.21 %	AutoGenerated7
6	0.72 Rf	1.9 AU	0.77 Rf	74.0 AU	17.66 %	0.80 Rf	27.7 AU	2220.6 AU	17.78 %	AutoGenerated3
7	0.81 Rf	29.4 AU	0.85 Rf	55.5 AU	13.24 %	0.89 Rf	0.7 AU	2112.1 AU	16.92 %	AutoGenerated4

Figure 5: HPTLC peak table of *Nishatipal* freeze dried powder

The qualitative phytochemical screening for the primary metabolites of the freeze-dried powder of *Nishatipal* (Table 4) revealed that it contained traces of proteins and reducing sugars with no starch. The qualitative phytochemical screening for the secondary metabolites of the freeze dried powder of *Nishaipal* (Table 5) revealed that it contained high content of phenolics, saponins, flavonoids and tannins, moderate content of alkaloids, terpenoids, monoterpenes, sequiterpenes and absence of steroids.

The comparative phytochemical screening of the both (Table 4 and Table 5) gave similar results for primary and secondary metabolites confirming the presence of similar compounds in both the decoction and the freeze-dried powder. This suggested very low change in chemical properties with the liquid decoction transformed to the solid form. Thus, it suggested the suitability of the freeze dried powder of *Nishatipal* to be used as an easy to use form of the NT decoction without changing the chemical properties and the efficacy.

The *invitro* antioxidant activity analysis was done through analyzing the total phenolic content and DPPH radical scavenging activity. The results of analysis of total phenolic content (298.01 ± 3.99 mg gallic acid equivalents /g of the extract) and DPPH radical scavenging activity (1020.93 ± 16.99mg trolox equivalents / g of extract and Trolox IC_{50} = 6.37 ± 0.01 µg/ml) indicating the very high antioxidant capacity of the NT freeze dried powder. Antioxidants may act at different levels to reduce oxidative stress, by inhibiting the formation of ROS (reactive oxygen species),by scavenging free radicals or by increasing the antioxidants defense enzyme capabilities[24,25,26].As oxidative stress is the main contributing factor towards the development of macrovascular and microvascular complications associated with diabetes, the management of oxidative stress leads to a potential management of the complications associated with diabetes.

In conclusion, quality control parameters were established for *Nishatipal* decoction and its freeze-dried form for the first time. In addition, antioxidant potential was evaluated for the freeze-dried powder of *Nishatipal* decoction in terms of total phenolic and flavonoid contents and DPPH scavenging assay.

Acknowledgements

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Evaluation on ethno-medicinal importance and conservation of medicinal plant *Monochoria vaginalis*

S. N. L Narathota[1]*, A. P. A. Jayasiri[1]

Absstract

Monochoria vaginalis (*Diya habarala*) of family PONTEDERIACEAE is an aquatic plant native to Sri Lanka with many ethno-medicinal values. This plant is used to treat numerous diseases in traditional system of medicine and used as a vegetable in some parts of the country. At present this has been included to lower risk category of IUCN Red Data Records due to reduction from its natural habitat. This study was conducted to evaluate ethno-medicinal importance, propagation and conservation of this plant. Data gathered from literature surveys, survey studies, physical parameter analysis tests and cultivation methods were utilized in this study. Illegal wetland destruction, excessive use of non-selective weedicides, climatic changes have minimized the availability of this plant. As a result, use of correct plant for drug preparation has reduced. Therefore, proper identification of this plant is also a necessity today. *Diya habarala* is a main ingredient of *Neelyaadi oil* which is used in traditional system of medicine. Cold potency of this plant reported to be one of the main Ayurveda pharmacological properties that helps in pacifying vitiated *Pitta Dosha*. Drug recipes including *Diya habarala* mainly act on integumentary system and used to treat skin rashes, ulcers, wounds and skin malignancies. Cultivation using Peat soil mixture showed good results and Tissue culture techniques can be used in conservation of this plant in future.

Keywords: Distribution, Ethno-medicinal values, Identification, *Monochoria vaginalis*, Propagation

Introduction

Monochoria vaginalis (*Diya habarala*/ Pond weed) of family PONTEDERIACEAE is an annual aquatic plant native to Sri Lanka and many other Asian countries. This is found in slow moving or standing water bodies such as border of tanks, near paddy fields, ponds and rivers[1]. This monocotyledonous plant grows about 10-30cm tall with shiny appearance and a short rhizome.

Ethno-medicine refers to range of healthcare systems, practices, remedies and therapeutic techniques that arise from indigenous cultural development[2]. Use of herbs to treat a variety of different ailments is universal and exists in every human culture. *Diya habarala* plant is a medicinal plant used in traditional system of medicine in Sri Lanka and used as a dietary material by native people. This study is carried out to identify the correct plant species, ethno-medicinal importance, risk factors for reduced availability and conservation methods.

Some texts mention that '*Jabara*' is a synonym for *Diya habarala*[3] whereas some texts mention that '*Jabara*' and *Diya habarala* are two different plant varieties. There's a controversy between *Diya habarala* and *Diya beraliya* plant too. Even though this plant is not commonly mentioned as a drug ingredient in Ayurvedic texts, it can be found in many traditional texts. For an example it's well known for anti-poisonous and wound healing action in traditional system of medicine. *Diya habarala* is a main ingredient of *Neelyaadi oil* which is used in fracture healing and wound healing treatments in traditional system of medicine in Sri Lanka[4].

This plant which has been abundantly seen in low country wet zone has shown reduced availability at present due to many reasons such as excessive usage of agro chemicals[5]. According to IUCN Red Data Records *Monochoria vaginalis* is classified under lower risk category and included in least concern version of its criteria[6]. Nowadays Ayurvedic practitioners and drug manufacturers face dearth of this plant for medicinal purposes as well. Unawareness of people about the value of this plant is a main reason for degradation of natural habitat of *Monochoria vaginalis*.

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Identifying the reasons for being reduced in natural habitat and finding innovative and effective methods to improve cultivation of this plant has become a need today. Present study attempted to analyze the medicinal importance, nutritional value and conservation methods of *Monochoria vaginalis*.

Materials and methods

Literature study

Literature study was carried out in order to identify morphological characteristics, ethno-medicinal value of *Monochoria vaginalis* in Ayurvedic and traditional medical texts. Data from various websites and research articles were also collected.

Field visits

Field visits were carried out to find distribution, availability and ethno-medicinal importance. Plants were authenticated at Department of *Dravyaguna Vignana,* Institute of Indigenous Medicine, University of Colombo and Herbarium sheets were prepared using *Monochoria vaginalis* specimens collected from natural habitats[7].

Survey study

Surveys were conducted among Ayurveda doctors, traditional practitioners, general public and paddy farmers using questionnaires to gather data on medicinal and nutritional importance, reasons for reduce availability and conservation methods of *Monochoria vaginalis*. Three types of questionnaires were prepared in both languages and distributed among these populations to gather data (Figure 1).

Tests to analyze physical parameters and chemical nature

Tests to analyze physical parameters and chemical nature[8] were conducted using dried powder of *Monochoria vaginalis.* Tests were conducted at Department of *Dravyaguna Vignana*, Institute of Indigenous Medicine, University of Colombo. Raw plant material (3kg) was washed and air dried for 5 days and 900g of powder was obtained after grinding.

Litmus test

Monochoria vaginalis powder (10g) was dissolved in distilled water and separated the mixture into 3 parts. Red litmus paper and blue litmus paper was put into 2 containers separately at the same time. Litmus papers were dipped and after about 30 seconds, took out of containers. Then the colour changes were observed.

Determination of pH value

Measured *Monochoria vaginalis* powder (60g) and made a bolus by covering with a cotton cloth. Then 8 cups (1920ml) of water was added to a fresh clay pot and dipped the bolus and boiled till water volume reduced to 1 cup (240ml). Calibrated the pH meter by measuring the pH of distilled water. After letting the decoction get cooled, checked the pH value using the pH meter[8].

Determination of moisture content

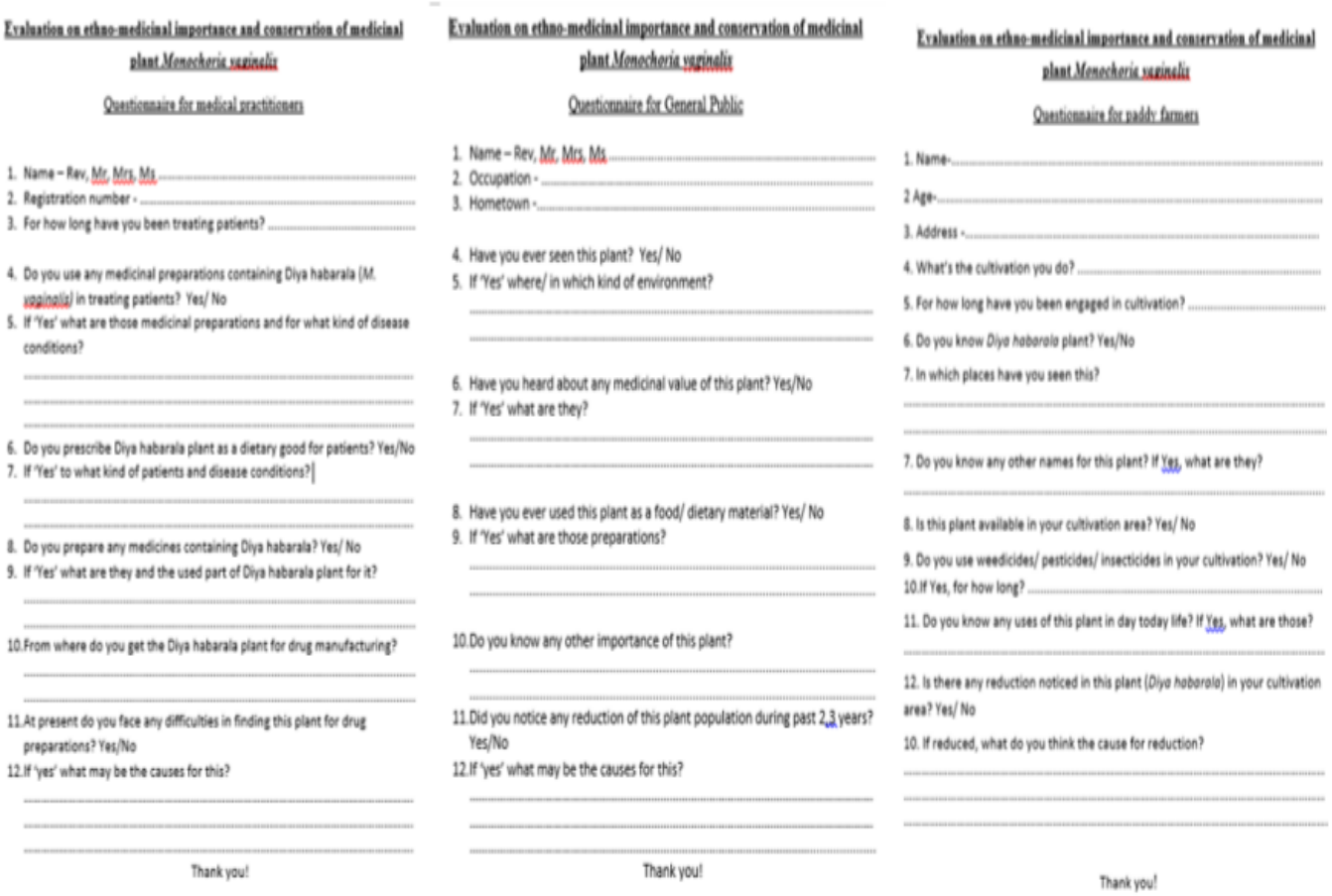
Monochoria vaginalis dried powder (2g) was measured and detected the moisture content using moisture apparatus in room temperature. 3 samples were checked and mean was calculated[9].

Determination of total Ash value

For this test 6 porcelain crucibles were washed, dried in hot plate and put 2.5g of *Monochoria vaginalis* powder to each of it. Then placed the 6 crucibles in desiccator and kept in the muffle furnace. Heated up to temperature of 550^{o}c till it became white/carbonized, cooled and weighed the samples[10].

Thin Layer Chromatography

Dried *Monochoria vaginalis* powder (20g) was measured and put into flat bottom flask and added 100ml of Ethanol. Mixed it well and covered the top with an Aluminium foil. Then placed it in the shaker for 24 hours, at the speed of 125rpm. After 24 hours, filtered the solution to a flask using folded filter papers. Then filtrate was changed to round bottom flask of rotary evaporator (Buchi r 3 Switzerland). Solution was taken out and condensed by placing it in a water bath of condenser. Concentrated solution was spotted on 10x10cm TLC plate (Aluminium sheet coated with normal phase silica gel 60, F_{254}) and TLC plate was placed in the solvent tank using Methanol: Hexane (8:2) as the mobile phase. After development, TLC plate was removed from TLC tank and allowed to dry. TLC plate was observed under UV light of 254nm wavelength using an UV cabinet (CAMAG Sr. N. 21317, Switzerland). Viewing TLC plates under UV light is non-destructive (compound is unchanged after process) while using chemical stain is destructive[11].



Evaluation on ethno-medicinal importance and conservation of medicinal plant *Monochoria vaginalis*

Questionnaire for medical practitioners

1. Name – Rev, Mr, Mrs, Ms .....
2. Registration number - .....
3. For how long have you been treating patients? .....
4. Do you use any medicinal preparations containing Diya habarala (*M. vaginalis*) in treating patients? Yes/ No
5. If 'Yes' what are those medicinal preparations and for what kind of disease conditions?
6. Do you prescribe Diya habarala plant as a dietary good for patients? Yes/No
7. If 'Yes' to what kind of patients and disease conditions?
8. Do you prepare any medicines containing Diya habarala? Yes/ No
9. If 'Yes' what are they and the used part of Diya habarala plant for it?
10. From where do you get the Diya habarala plant for drug manufacturing?
11. At present do you face any difficulties in finding this plant for drug preparations? Yes/No
12. If 'yes' what may be the causes for this?

Thank you!

Evaluation on ethno-medicinal importance and conservation of medicinal plant *Monochoria vaginalis*

Questionnaire for General Public

1. Name – Rev, Mr, Mrs, Ms .....
2. Occupation - .....
3. Hometown - .....
4. Have you ever seen this plant? Yes/ No
5. If 'Yes' where/ in which kind of environment?
6. Have you heard about any medicinal value of this plant? Yes/No
7. If 'Yes' what are they?
8. Have you ever used this plant as a food/ dietary material? Yes/ No
9. If 'Yes' what are those preparations?
10. Do you know any other importance of this plant?
11. Did you notice any reduction of this plant population during past 2,3 years? Yes/No
12. If 'yes' what may be the causes for this?

Thank you!

Evaluation on ethno-medicinal importance and conservation of medicinal plant *Monochoria vaginalis*

Questionnaire for paddy farmers

1. Name- .....
2. Age- .....
3. Address - .....
4. What's the cultivation you do? .....
5. For how long have you been engaged in cultivation? .....
6. Do you know *Diya habarala* plant? Yes/No
7. In which places have you seen this?
7. Do you know any other names for this plant? If Yes, what are they?
8. Is this plant available in your cultivation area? Yes/ No
9. Do you use weedicides/ pesticides/ insecticides in your cultivation? Yes/ No
10. If Yes, for how long? .....
11. Do you know any uses of this plant in day today life? If Yes, what are those?
12. Is there any reduction noticed in this plant (*Diya habarala*) in your cultivation area? Yes/ No
10. If reduced, what do you think the cause for reduction?

Thank you!

Figure 1: Samples of questionnaires distributed among Ayurvedic Doctors, farmers and general public in survey study

High Performance Thin Layer Chromatography

This technique is widely employed in identification, detection of adulterants in herbal product which helps in identification of pesticide content and in quality control of herbs[12]. This has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. TLC plate was placed in HPTLC analyzer and results were analyzed.

Cultivation and Propagation

Plants were cultivated in 2 different environments and growth was observed and records were taken for 3 months.

- 5 plants were cultivated in an open area near water drainage at home garden. Watered the plants daily.
- 5 plants were planted in 5 pots in University premises. Initially compost soil was used and then added peat soil[13, 14]. Watered the plants daily.

Results

Identification

Literature surveys revealed multiple data on the morphological features for correct identification, vernacular names, distribution and nutritional importance of *Monochoria vaginalis*.

In Sinhala, this is known as *Diya habarala, Diya beraliya, Diya habaru*[3] whereas in Sanskrit *Indeevarah* [15] and *Indeevar*[16]. In English, this is known as Oval leaf pond weed, Pickerel weed, Water hyacinth and in Tamil as *Karim kuwalam* [17], *Karun kuwalai* [15]. *Nanka* [15] is the commonest name in Hindi language for this plant.

Macroscopic identification of plant species was mainly conducted by analyzing morphological features [18] (Table 1) and prepared Herbarium sheets of *Monochoria vaginalis* (Figure 2).

Table 1: Morphological features of *Monochoria vaginalis*

Morphological Feature	Description
Habit	Smooth, fleshy, tufted, annual or perennial aquatic herb about 10-50 cm tall with a glabrous, shiny appearance and a short rhizome
Stem	Inconspicuous, obliquely erect.
Roots	Fibrous at base of petioles. Submerged under water or rooted in the mud. Very short, thick, spongy and purplish green branched rhizome.
Leaves	Size and shape is highly variable. In young plants without lamina, leaves are 2-12.5 cm long and 0.5-10 cm wide and narrow at early stage. In older plants broadly ovate, sharply acuminate with a heart-shaped or rounded base, shiny, deep-green with longitudinal veins.
Inflorescence	Spicate, 3-6 cm long. Spike-like inflorescence opposite the floral leaf, and one stamen with a lateral, oblique, erect tooth. Inflorescences bend downward is special.
Flowers	Pedicelled, bisexual, Opposite the sheath of floral leaf at base, violet or lilac blue in colour. [6] Flowers number from 3-25 and open simultaneously or from top to bottom in quick succession. Flowering: - March – August
Fruits	Capsule of about 1cm diameter, splitting between the partitions into three valves. Fruit may mature below the water surface.
Seeds	Propagated by seeds and vegetative parts. Elliptic to slightly ovate, about 1 mm long and raphe sometimes present as a prominent longitudinal ridge.

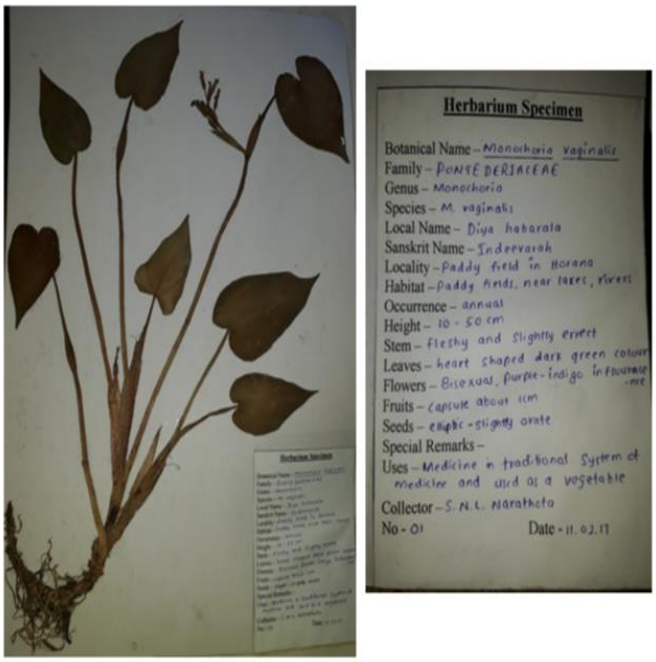


Figure 2: Herbarium sheet of *Monochoria vaginalis* prepared using a specimen collected from a paddy field in Horana, Kalutara district

Distribution

Data on distribution and availability were collected from literature studies and survey studies. This is an annual plant that grows as a perennial in constantly flooded areas. Wide distribution in South East Asia and commonly seen in countries like Philippines, China, Korea, Vietnam, Bhutan, Cambodia, India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, Taiwan and Sri Lanka[19] In Sri Lanka this grows near paddy fields, riverbanks and lakes especially in low country. This can be seen abundantly in areas such as Rathnapura, Badulla, Makandura, Kegalle, Gampaha, Matale, Galle, Kalutara, Mathugama and Horana. Collected data was interpreted on map [20] indicating 3 climatic zones of Sri Lanka. (Figure 3) It is often abundant in stagnant backwaters or rivers, in open drains, rice fields, swampy places, ditches and wet pastures[19].

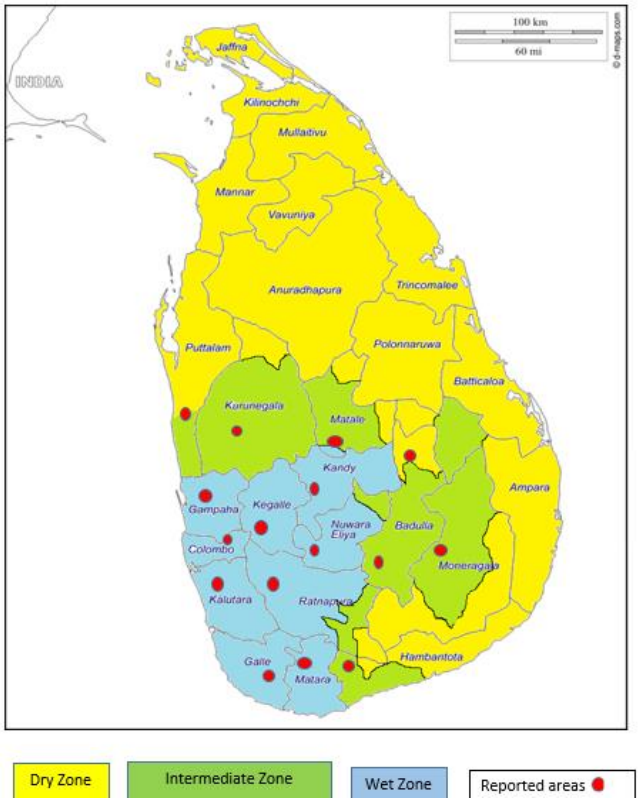


Figure 3: Data collected on distribution of *Monochoria vaginalis* illustrated on a map of Sri Lanka with different climate zones

Ethno - Medicinal importance

Details mentioned in Ayurveda pharmacopoeias and traditional medical texts about the pharmacodynamic properties of *Monochoria vaginalis*

Rasa (Taste): *Madhura* (Sweet)
Guna (Properties): *Laghu* (Lightness)
Veerya (Potency): *Sheetha* (Cold)
Vipaka (Final digestive transformation): *Katu* (Pungent)

Used parts: *Svarasa* or Water extraction / fresh juice of whole plant and leaves [4]

Therapeutic importance supported by clinical data

Leaf juice of *Monochoria. vaginalis* is used to treat cough, asthma, toothache, stomach and liver problems. Fraction of n-butanol from *Monochoria. vaginalis* exhibited anti-oxidant activity and root extracts were evaluated for anti-inflammatory and anti-nephrotoxic activities. Tail immersion and hot plate studies of alcoholic extract of *Monochoria vaginalis* had showed significant analgesic activity. Effective in preventing liver fibrosis, cirrhosis and hepato-carcinogenesis [21]

Disorders that can be cured with drug recipes including *Diya habarala* [22]

GIT: Constipation, Gastritis, Ulcerative colitis, Indigestion, Dyspepsia, Scurvy, Hepatic ailments, Piles, Hemorrhoids, Rectal prolapse
Respiratory system: Asthma, Cough
Circulatory system: Anemia, Blood poisoning
Integumentary system: Skin disorders such as rashes, wounds, ulcers
Genitourinary tract: Anuria, Dysuria, burning sensation in urinating, Menorrhagia
Endocrine system: Diabetes[4] (Figure 4)

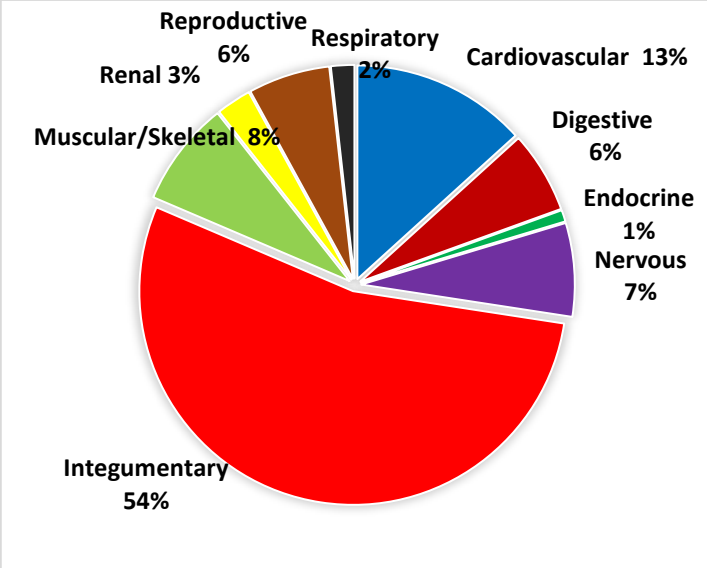


Figure 4: Percentage of drug recipes including *Monochoria vaginalis* stated in *Thalpathe Piliyam* book series according to target body system

Multiple home remedies including *Monochoria vaginalis* were collected by survey study (Table 2). Integumentary system includes skin and its appendages acting to protect body from various kinds of damage from outside. This includes hair, gland and nerves. *Neelyaadi thailaya* is a commonly used oil in Sri Lankan traditional medicine which acts against diseases of integumentary system. This oil is well known for wound healing, fracture healing, lowering blood pressure, headaches and skin diseases. Although there are different drug recipes for preparing this oil, *Diya habarala* is included as a main ingredient in all those recipes[4, 23].

Table 2: Home remedies using *Monochoria vaginalis* found in survey study

Disease Condition	Treatment
Constipation	Prepare gruel with rice and drink. Apply grinded paste around anus in small children.
Urinary calculi	Grind the plant and eat with ghee.
Toothache	Keep grinded plant on painful tooth.
Burning sensation of body	Eat raw salad prepared using this plant.
Hemorrhoids	Eat curry prepared with these leaves.

Nutritional and other importance

All parts of *Monochoria. vaginalis* except the roots are relished as a vegetable[3]. It's mentioned that this plant can be used as an alternative food to alleviate protein–energy malnutrition in populations in developing countries[24]. Carbohydrate, protein and phosphate levels in tissues were similar in leaves and flowers which indicate that both leaves and flowers have nutritional properties equally. There were differences in mineral and protein levels in leaves and flowers of *Monochoria vaginalis*. Amount of non-essential amino acids especially aspartic and glutamic acids were higher in flowers than leaves. Minerals like calcium, potassium, magnesium also found in this plant which helps in gaining adequate nutrition. Anti-nutritional compounds like phenol and tannins had also found in nutritional assessment[24]. Organic compounds of plant origin have pronounced physiological actions on humans. Remarkable presence of organic compounds like alkaloids, flavonoids, glycosides in this plant proves the nutritional value of this plant well[25]. Some food recipes prepared using *Monochoria vaginalis* with medicinal importance were collected in this study (Table 3).

It has also said that this provides a friendly environment to fish and other animals living in water and has found that areas where *Monochoria vaginalis* is more the fish population is also higher[26]. Other than above mentioned benefits this plant is used as cattle fodder in countries like Indonesia[20].

Physical parameter analysis

Litmus test

Red litmus paper showed no change and blue litmus has turned to light pinkish colour, which indicates that *Monochoria vaginalis* solution was having acidic properties.

Determination of pH value

pH value of decoction of *Monochoria vaginalis* was tested and repeated the test for 3 times and the average value was taken. pH value of decoction of *Monochoria vaginalis* = 5.87

Determination of moisture percentage

Moisture content of crude drug is directly related to its stability when there are chances of microbial growth. The shelf life of the drug also increases with lowering the moisture contents. Moisture value of 2g of *Monochoria vaginalis* = 12.12%.

Determination of total Ash value

The total Ash value was 15.23% with a standard deviation of 2.6154. Standard deviation is used to tell how the measurements of a set of results are spread out from the average (mean), or expected value[27]. Depending on the level of contaminations total Ash value can be varied[10].

$$\text{Total Ash Percentage} = \frac{\text{Weight of total ash}}{\text{Weight of raw powder}} \times 100\%$$

$$= (14.96+18.08+10.16+16.84+14.24+17.12)/6$$

$$= 15.233\%$$

Table 3: Common food recipes found from survey study including *Monochoria vaginalis*

Food Recipe	Method of Preparation
 ***Diya habarala kola maaluwa***	*Diya habarala* leaves are well washed and cut. Add some onions, raw chillies, curry leaves, powder of Maldive fish (*Umbalakada*), salt, turmeric powder, chillie powder and cook it on fire. Temper with ghee. Taste can be further increased by adding cooked dhal or jack seeds.
 **Tempered *Diya habarala* leaf curry**	Tender *Diya habarala* leaves and stems are washed well and cut into pieces. Add some onions, raw chillie, *Umbalakada*, turmeric, curry powder, salt and mix well. Temper the curry with coconut oil.
 ***Diya habarala kola malluma***	*Diya habarala* leaves are washed well and cut into small pieces. Add some onions, raw chillie, curry leaves, salt, *Umbalakada* and cook on low fire. Mix scraped coconut, turmeric powder. Temper with oil. Effective for hemorrhoids.
***Diya habarala* raw salad**	*Diya habarala* leaves are washed well and cut into small pieces and onion, raw chille, *Umbalakada* powder and salt is mixed.

Thin Layer Chromatography

Thin layer chromatogram of ethanol extract was observed from *Monochoria vaginalis* powder. Solvent system used was Methanol: Hexane, 8:2 and after development, visualized 2 spots under UV light of 254nm wavelength (Figure 5).

High Performance Thin Layer Chromatography

An extension of TLC is high performance thin layer chromatography (HPTLC) which is robust, simplest, rapid, and efficient tool in quantitative analysis of compounds. Obtained 5 RF values in this test which showed the presence of chemical compounds (Figure 6).

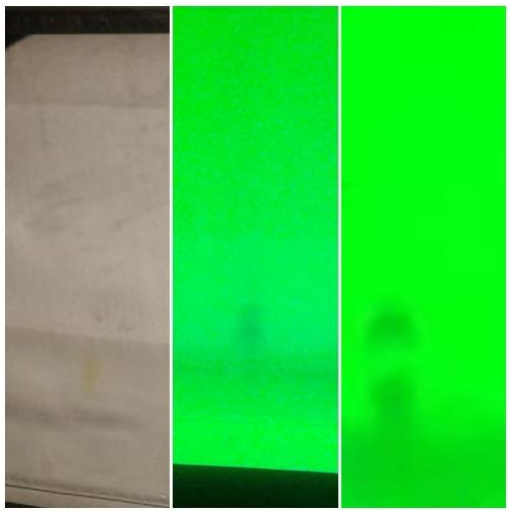


Figure 5: Results of Thin Layer Chromatography visualized under 254nm UV wavelength

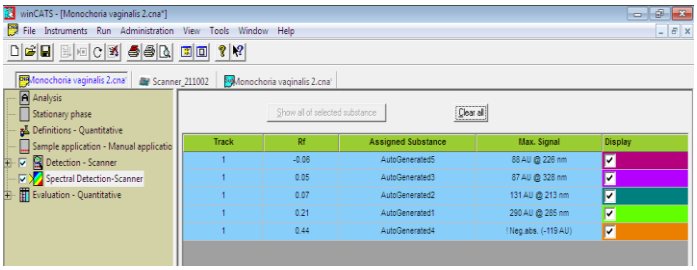


Figure 6: Results of High-Performance Thin Layer Chromatography

Reasons for the lack of availability

- Heavy usage of agro-chemicals such as non-selective weedicides in paddy cultivation
- Illegal wetland destruction
- Land reclamation for development purposes
- Environmental pollution
- Climatic changes
- Difficulty in competing for basic requirements like sunlight, air, apace, soil with plant species like moss/*Paasi* (plants belonging to division Bryophyta) and other invasive plants like *Japan jabara*
- Destruction as a result of unawareness of people about the importance of this plant
- Hardness of water
    - ➢ Main cause for reduction in North Central province in Sri Lanka
- Salty wind and sandy soil
    - ➢ Main reasons for reduction in coastal margin of Sri Lanka

Cultivation and Propagation

Plants were cultivated in 2 different environments and observations were recorded for 3 months.

Plants cultivated near water drainage

Height of plant, number and size of leaves had increased. Three plants had flowers over the past 3 months and flowering occurred well during March and April. Plants grew well (Table 4).

Plants cultivated in clay pots

Initially cultivated in clay pots with compost soil and after 1 month plants started to become weak, smaller in size and the number of leaves also got reduced. Then the Peat soil [27] was prepared and added. Watered the plants daily. After about a week plants started to grow well. Flowering was mostly in March. At the end of 3 months only 3 plants had liveliness and healthy appearance. Overall, the growth was successful compared to compost soil mixture (Table 5).

Discussion

Monochoria vaginalis is an annual aquatic monocotyledonous plant which belongs to Order Liliales, Family PONTEDERIACEAE and Genus *Monochoria C. presl* (*Monochoria*). *Diya habarala* is known as Diya habaru, Diya beraliya and Jabara. Jabara is a vernacular name for *Japan jabara* (*Eichchornia crassipes*) too. Even though *Eichchornia crassipes* [28] belongs to Family PONTEDERIACEAE, Genus differed as *Eichchornia*. This is an invasive plant with no specific ethno-medicinal value and propagated by runners and destroys natural habitat of many aquatic plants.

Low country wet zone recorded the highest population of *Monochoria vaginalis* and at present it has showed drastic reduction in areas where the use of weedicides is higher. Non selective weedicides, illegal wetland destruction, climatic changes are the main reasons for the lack of availability of this plant in natural habitat and has resulted in inclusion of this plant under lower risk category of IUCN Red Data Records.

By this study, it was cleared that this plant has important ethno medicinal values. Other than the medicinal importance this has nutritional value and used as a vegetable in many parts of the country by native people. Commonly the leaves are used as a vegetable because of the higher nutritional values. It has also mentioned that this plant can be used as a food supplement in protein energy malnutrition for people in developing countries. *Diya habarala* is used in many drug recipes of traditional system of medicine and *Neelyaadiya* oil is one of the widly used drugs prepared using this plant. Most of other drug recipes are used for treating wounds (*Gadu*, *Odu*), skin malignancies and other disease conditions of Integumentary system. This plant is used in treating different ailments such as anuria and constipation in day today life. Most of the therapeutic values of this plant are due to its cold potency. This helps in pacifying and alleviating vitiated *Pitta dosha*.

Monochoria vaginalis decoction was Acidic with a pH of 5.87. Decoction was used as the extraction of chemicals was higher and powder didn't dissolve well in water. Increased moisture was a proof to assume the reduced shelf life of this drug powder. Total Ash value was tested in this study. Ash value is useful in determining authenticity and purity of sample and these values are important in qualitative standards.

Table 4: Records on plants cultivated near water drainage

16.01.17	06.02.17
	
5 plants were planted near water drainage in home garden	After 2 weeks height has increased a bit. No any other significant observations
06.03.17	13.03.17
	
Small flowers were observed	Height and leaf size also increased
27.03.17	03.04.17
	
Height and number of leaves have increased	Plants have well adapted to natural environment. Height and average size of leaves have increased. Fully grown flowers were observed

Table 5: Records on plants cultivated in clay pots

10.02.17  5 plants were cultivated in clay pots	22.02.17  Roots have become fixed. Average height was nearly 12cm. Average breadth of leaves was around 4cm
06.03.17  One plant has shed all the leaves and has started to become weak. Others had no change in number or size of leaves.	Peat Soil was added  
27.03.17  Weakened plants has got fresh with increased height, size and number of leaves	07.04.17  Fully grown flowers were observed in all 5 pots
25.04.17  Flowers were observed in all pots.	15.05.17  Plants have become thin and dried. One plant has died. Environmental temperature has increased to 32ºC

By TLC and HPTLC studies, 5R_f values were obtained which revealed the presence of chemical groups such as Alkaloids, Tannins and Glycosides that may be accountable for therapeutic effects in treating above mentioned diseases.

Sufficient water supply, space, sunlight and moisture were essential for these plants to grow well. Plants cultivated in pots showed successful growth after adding peat soil mixture and started flowering. Peat soil was prepared using soil collected from a paddy field which was similar to their natural habitat. Peat forms when plant material does not fully decay in acidic and anaerobic conditions. It is mainly composed of wetland vegetation including mosses and shrubs. As it accumulates, peat holds water[13]. Peat soil which was prepared using decaying organic components was effective in cultivation and propagation of this plant and can be used in conservation and commercial cultivation. [14] Even a slight change in climate can severely affect the growth of *Monochoria vaginalis* mainly when they are not in natural habitat. These plants propagate from seeds and vegetative parts in a way much similar to propagation of aquatic plants.

Conclusion

Monochoria vaginalis (*Diya habarala*) is an annual aquatic monocotyledonous plant with many ethno-medicinal values. This drug is mainly used in Traditional system of medicine for diseases of Integumentary system and reported to have anti-oxidant activity. At present this has been included in IUCN Red Data Records due to degradation of its natural habitat. This is used as a vegetable in many areas and can take necessary steps to encourage the use of this nutritious plant as a food article. Cultivation using peat soil mixture showed the best results in propagation. Further analysis through isolation of chemical constituents and structure elucidation can be used in development of new drugs which can be beneficial in treating above mentioned diseases. Micro-propagation methods such as Tissue culture techniques can be used for conservation and commercial cultivation of this plant for future.

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Determination of *Mizaj* (Temperament) in women with mixed urinary incontinence: A preliminary study

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Abstract

Mixed urinary incontinence (MUI) has been defined symptomatically by the International Continence Society as ‘‘the complaint of involuntary leakage associated with urgency and also with exertion, effort, sneezing or coughing.’’ One of the fundamental concepts of Unani system of medicine is *Mizaj*. *Mizaj* of the organ/body may be affected by internal and external factors leading to *Su’-i-Mizaj*, causes disease. Some of the causes of *Salas al-Bawl* are *Su’-i-Mizaj Barid, Zo’fe Mathana, Mudirr-i-Bawl, Khala Faqra,* alcohol, excessive fluid intake, or injury to *Adala al-Mathana*. Therefore, evaluation of *Mizaj* of an individual or organ is important for treatment of the disease. Thus, this study was conducted to determine the *Mizaj* in women with MUI. A prospective, single centre preliminary study was conducted in 60 women diagnosed with MUI from February 2015 to June 2015 at the National Institute of Unani medicine, India. Questionnaire for assessment of Women’s general *Mizaj* (based on *Alamat AjnaseAshra*) was used. Further, *Alamat* Su’-*i-Mizaj* (clinical features of abnormal temperament) of body as described in the traditional Unani literature were used to assess the *Su’-i-Mizaj*. The data was analyzed by descriptive analysis using Graph pad. Of 60 patients, 43(71.66%), 2(3.33%) and 15(25%) patients had *Barid, Harr* and *Motadil* general *Mizaj* respectively. Of 60 patients, 13(21.66%), 25(41.66%) and 22(36.66%) patients had *Yabis, Ratb* and *Motadil* general *Mizaj* respectively. Of 60 patients, 56(93.33%), and 4(6.66%) patients had *Barid,*and *Harr Su’-i-Mizaj* respectively. This preliminary study validated the claim of Unani scholars that this disease is more common in *Barid Mizaj* and the *Su’-i-Mizaj* is towards *Burudat* in women with MUI.

Key words: *Akhlat*, *Mizaj*, Mixed urinary incontinence, *Salas al - Bawl, Su’- i - Mizaj*

Introduction

World Health Organization (WHO) defines health as the “condition of total physical, emotional and social health and prosperity” [1]. Urinary incontinence (UI) is defined by the International Continence Society as the "complaint of any involuntary leakage of urine". Urinary incontinence is not life threatening however; it is associated with significant reduction in health related quality of life (HRQoL)[2] and at the same time has additional financial burden. Urinary incontinence is increasingly seen because of its high prevalence (20% to 30% of middle-aged and 30% to 50% of elderly women) and the growing expectations for relief by women affected by it[3]. The most common types of female UI are stress, urge and mixed incontinence. Mixed urinary incontinence (MUI) has been defined symptomatically by the International Continence Society as ‘‘the complaint of involuntary leakage associated with urgency and also with exertion, effort, sneezing or coughing.’’[4] Mixed urinary incontinence (MUI) is the presence of both SUI and UUI symptoms[5]. It is linked to concomitant disturbances, which may be due to childbirth, aging, or other medical conditions, in the complex bladder-urethra coordinated system of urine storage and emptying[6]. MUI accounts for approximately 33% of all cases of incontinence in women[7].

One of the fundamental concepts of Unani system of medicine is *Mizaj*. *Mizaj* of the organ/body may be affected by internal and external factors leading to *Su’-i-Mizaj*, causes disease[8]. The principle of management of disease is to correct the altered temperament.

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Therefore, before commencing any treatment, *Mizaj* of a patient or organ has to be evaluated. Unani classical literature defines *Salas al-Bawl* or urinary incontinence as involuntary loss of urine[9, 10]. The causes of *Salas al-Bawl* as per Unani classical texts are *Istirkha Adala al-Mathana* (laxity of muscular layer of bladder), which surrounds the neck of the bladder or duct causes *Salas al-Bawl*. *Salas al-Bawl* is also caused due to *Khala Faqra* (dislocation of vertebrae), *Khal'al-Mathana* (dislocation of bladder) *Istirkha Ribat al-Mathana* (laxity of ligaments of bladder)[11]. *Su'-i-mizaj Barid, Du'fe Mathana*, *Mudirr-i-Bawl, Khala Faqra,* alcohol, excessive fluid intake, or injury to *Adalaal-Mathana,* diseases of surrounding structures such as *Waram al-Rahim* (endometritis/PID), *Waram al-Sura* (omphalitis), constipation, and *Haml* (pregnancy)[12]. *Du'fe* and *Istirkha Mathana* is caused because of excessive intake of cold and moist things.[13] *Rutubat* in the *Mathana* leads to *Du'fe Quwwat-i-Masika,* henceforth, *Salas al-Bawl* is frequently seen in children. *Taqtir al- bawl* (dribbling of urine) occurs because of *Istirkha Adala al-Mathana* and *Du'fe Mathana* (weakness in bladder) or *Hiddat al-Mathana.*[14] One of the causes for *Salas al-Bawl* is *Su'-i-Mizaj*. Hence, *Mizaj* of the patient in this disease should be assessed so that appropriate treatment can be given. Thus, this study was planned to determine the *Mizaj* in women with MUI.

Material and Methods

A prospective, single centre preliminary study was conducted in 60 women diagnosed with MUI at the National Institute of Unani medicine, India from February 2015 to June 2015. Both written and oral information about the reasons for this study were given to women and requested to participate. The ethical number of the study is IEC No: NIUM/IEC/2013-14/015/ANQ. Parous women aged ≥21 years with symptoms of MUI as evidenced by stress and urge symptoms reported on MESA (Medical, Epidemiologic and Social Aspects of Aging) questionnaire were included. Women with known systemic and endocrine diseases such as uncontrolled hypertension, diabetes mellitus, bronchial asthma, known malignancies, pregnant and lactating women were excluded.[15]

Patients were diagnosed with MUI based on stress and urge symptoms reported on MESA questionnaire. Researcher collected relevant socio-demographic data, clinical information and conducted general physical and gynecological examination with cough stress test. General, physical and systemic examination was conducted to exclude general and systemic diseases respectively. Mental status assessment included patient's orientation, level of consciousness and comprehension. Pelvic examination included observation for any vaginal or cervical discharge, full bladder and supine empty bladder cough stress test, pelvic floor muscular strength (PFMS),[16] vaginal wall, cervix, uterine size and genital prolapse assessment.

Validated *Mizaj* Questionnaire

Validated *Mizaj* Questionnaire for assessment of Women's general *Mizaj* (based on *Alamat Ajnase Ashra* discussed by Ibn Sina[10]) was used summarized in table 1. The questionnaire included 10 questions from 39 questions presented in *Alamat Ajnase Ashra.* Question 2 and Question 3 give the score of wet and dry scale 2 to 6, the dry score is ≥5 and wet is ≤3. Other questions include the score of warm-cold scale could be 8 to 24, warm ≥ 19, and cold ≤ 14. The weighted kappa coefficient of 20 questions were between 0.40-0.59, 18 questions were between 0.6-0.79 and one question was 0.83 for 39 questions of *Alamat Ajnase Ashra.* The Cronbach's α coefficient of this questionnaire was 0.71[17].

Alamat Su'-*i*-*Mizaj*

Alamat Su'-*i- Mizaj (*clinical features of abnormal temperament) of body as described in the traditional Unani literature were used to assess the *Su'-i-Mizaj* (Table 2).[10] Signs and symptoms were scored on rating scale 4 through 1 for *Alamat* Su'-*i-Mizaj*. Total score of each patient was added up and the inferences for type of su' *mizaj* was deducted based on equal interval scale developed from total score for the questionnaire. The reliability of the questionnaire was found to be 0.87 for split half reliability.[18]

MESA Questionnaire

MESA Questionnaire is useful to record urinary incontinence severity and incontinence subtype (stress or urge or MUI). The MESA is a self- reported questionnaire with nine questions on stress incontinence and six questions on urge incontinence. The four response categories ranged from "never" (0 points), "rarely" (1 point), "sometimes" (2 points) and "often" (3 points). The subscale scores are the sum of responses to the individual items with higher scores indicating more frequent symptoms of incontinence.

Table 1: Selected items for self-reported *Mizaj* Questionnaire

Selected items for Wetness or Dryness (Wet-Dry scale)[a]			
Question	**1**	**2**	**3**
Q1 When others touch your skin, what do they say about its warmness or coldness?	cold	not cold, not warm	very warm
Q2[b] How is the condition of your skin's Softness or dryness?	very soft	not soft, not dry	very dry
Q3[b] Are you fat or thin compared to others?	very fat	not fat, not thin	very thin
Q11 How big is the palm of your hand?	small	not small, not big	big
Q16 How fast are you influenced by Warmness or coldness?	I feel cold, fast	I feel the same in both cases	I feel warm, fast
Q17 How fast are you influenced by Warm nature foods as honey, spices, Paper or cold nature foods as buttermilk, yogurt and cucumber?	I feel cold, fast by cold nature foods	I feel the same in both cases	I feel warm, fast by warm nature foods.
Q20 How is your voice power compared to others?	weak	not weak, not strong	strong
Q24 How do you pronounce several consequent sentences?	articulate	not articulate, not continuous	continuous
Q25 How is your rage and anger?	I get angry late	I get angry no late no fast	I get angry fast
Q26 How is your physical movements compared to others?	very slow	not slow, not fast	fast

[a]The score of warm-cold scale could be 8 to 24. Warm ≥ 19, cold ≤ 14.
[b]The score of wet-dry scale could be 2 to 6. Dry ≥ 5, wet ≤ 3.

At pre-study screening the patient was placed in to 1 of the 4 categories on the basis of her response to the stress and urge incontinence items on the MESA measure: (1)pure stress incontinence, if the patient endorsed (answered affirmatively) only stress items; (2) pure urge incontinence, if the patient endorsed only urge items; (3) mixed urinary incontinence, if she endorsed both stress and urge items; and (4) continent, if the patient endorsed any incontinence items as "never" or "rarely". With the MESA instrument scored on 4 levels (continent, stress, urge or MUI), there was fair test-retest reliability (kappa, 0.39). Validity- a strong association was seen between incontinence characterization with the MESA and Hunskaar questionnaires.[19]

Table 2: Clinical features of *Alamate Su'-i- Mizaj*

***AlamateSu'-i-Mizaj* (Clinical features of Abnormal Temperament)**	
***su'–i-Mizaj Harr* (Warm)**	***Su'-i -Mizaj Barid* (Cold)**
☐ Feeling of uncomfortable heat ☐ Undue discomfort in fever ☐ Quick exhaustion of energy as activity flares up the heat ☐ Excessive thirst ☐ Weak quick and rapid pulse ☐ Burning and irritation in the pit of stomach ☐ Bitter taste in mouth ☐ Intolerance of hot foods ☐ Comfort from cold things ☐ Distress in hot weather	☐ Weak digestion ☐ Less desire for drinks ☐ Laxity of joints ☐ Tendency for catarrhal conditions and phlegmatic fevers ☐ Fondness for hot dishes and aversion of cold ones ☐ Greater discomfort in winters
***Su'-i-Mizaj Ratb* (Moisture)**	***Su'-i-MizajYabis* (Dryness)**
☐ Laxity ☐ Excess of salivation and nasal secretions ☐ Tendency towards diarrhea and dyspepsia ☐ Intolerance towards moist foods ☐ Excess of sleep ☐ Puffiness of eyelids	☐ Dry skin ☐ Insomnia ☐ Wasting ☐ Intolerance of dry foods but affinity for moist things ☐ Discomfort in autumn ☐ Ready absorption by the body of hot water and light oils

Data analysis

Statistical software

The Statistical software Graph Pad Instat version 3.00 for window (Graph Pad Software, San Diego, Calif,

USA) was used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

Statistical analysis

Descriptive analysis was performed by means of the frequencies of the category variables and measurements of the position and dispersion of the continuous variables. Results on continuous measurements were presented on Mean ±SD (Min-Max) and results on categorical measurements were presented in number (%).

Sample Size

Based on the range scores of the scales, the sample size was calculated as 60 by the Statistician.

$$Sample\ size = \left[\frac{\partial}{\mu 1 - \mu 2}\right]^2 = \left[\frac{6.0}{10 - 7.2}\right]^2 x\ 15 = 60$$

P =0.01

Informed Consent

Patients fulfilling the inclusion criteria mentioned above were given information sheet having details regarding the nature of study and written informed consent was obtained, if they agree to participate in the study**.**

Results

The age of 60 patients with MUI ranged from 21 to 60 years. The mean age was 40.91±7.92 years. Five (8.33%), 27(45%), 21(35%) and 7(11.66%) patients were in the age group of 21-30, 31-40, 41-50 and 51-60 years respectively. Out of 60 patients, 50(83.33%) were Muslims and 10(16.66%) were Hindus. All patients (n=60) were from urban area. Of 60 patients, the maximum no. of patients, 38(63.33%) were from the upper lower class followed by 21(35%), and 1(1.67%) in the lower middle and lower class respectively.

The mean BMI of patients was 26.85±3.1 kg/m^2. The mean duration of incontinence was 18±17.8 months. Of 60 patients, duration of incontinence was <12, 12-24 and > 24 months in 18(30%), 35(58.33%) and 7(11.66%) patients respectively. The mean score for urge urinary incontinence (UUI) and stress urinary incontinence (SUI) on MESA score was 15.24±2.14 and 25.9±1.65 respectively.

Of 60 patients, 43(71.66%), 5(3.33%) and 15(25%) patients had general *Mizaj* respectively (Figure 1). Of 60 patients, 13(21.66%), 25(41.66%) and 22(36.66%) patients had *Yabis*, *Ratb* and *Motadil* general *Mizaj* respectively (Figure 2). Of 60 patients, 56(93.33%), and 4(6.66%) patients had *Barid*, and *Harr Su'-i-Mizaj* respectively (Figure 3).

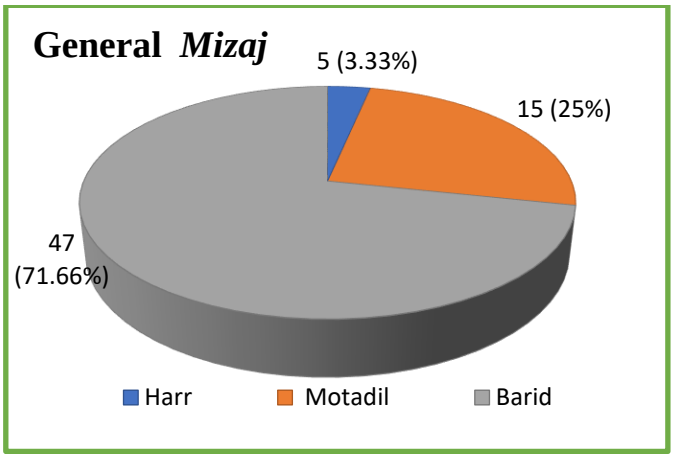

Figure 1: General *Mizaj* of *Barid, Harr* and *Motadil*

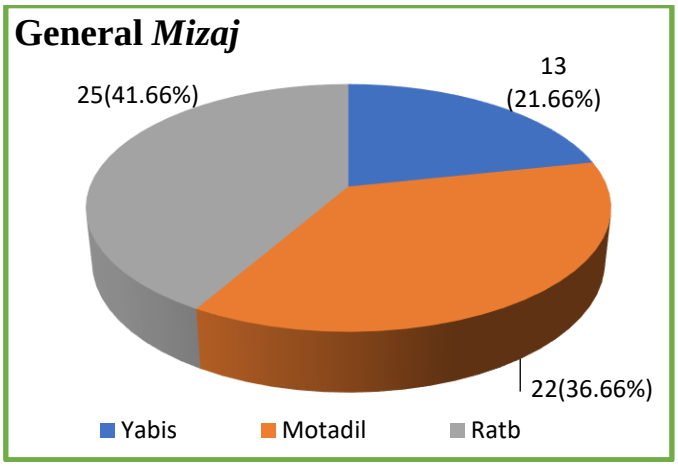

Figure 2: General *Mizaj* of *Yabis, Ratb* and *Motadil*

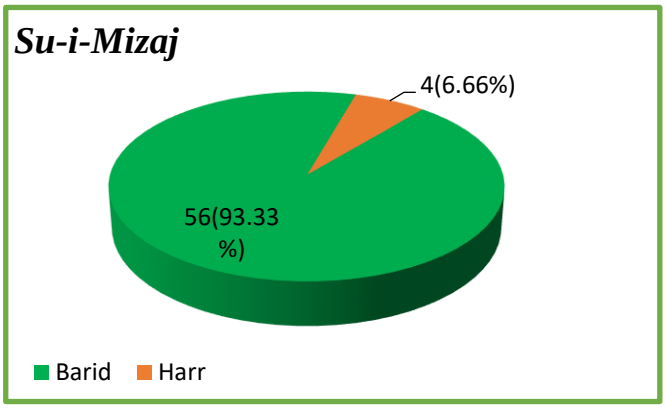

Figure 3: *Barid* and *Harr Su'-i-Mizaj*

Discussion

Age

It has been reported that that middle aged or older women with mixed incontinence were 2-3 times more likely to experience a greater quality of life impact than SUI.[20] The prevalence of incontinence appears to increase gradually during young adult life. A broad peak is noted at middle age and then steadily increases after age 65.[21]

Body Mass Index (BMI)

Weight loss has been shown to decrease UI in morbidly obese women.[22] BMI was associated with urge and mixed incontinence but not with stress incontinence. There may be a stronger association of increasing weight with prevalent and incident of stress incontinence, including mixed incontinence, than with urge incontinence and overactive bladder syndrome.[23] The women with MUI had slightly higher body mass index than the women with USI.[7] In a randomized controlled trial, 338 overweight and obese women with MUI were randomized to an intensive weight-loss program and behavior modification or to a structured education program. After 6 months women in the weight-loss program lost significantly more weight and had significantly fewer incontinence episodes weekly than those in the education group.[6]

Mizaj

Most of the patients had *Balghami Mizaj*. This finding confirms the writings of ancient Unani scholars that *Salas al-Bawl* is commonly seen in *Balghami Mizaj*. Ali bin Abbas Majusi opined that laxity of muscular layer of bladder which surrounds the neck of the bladder or duct causes involuntary loss of urine.[8] *Rutubat* in the *Mathana* leads *Du'fe Quwwat Masika*, henceforth lead to *Salas al-Bawl*.[10] Kabir al-Din[24] and Muhadhdhab al

Deen Al Baghdadi surmised that *Galaba Burudat al-Mathana*leads to *Salas al-Bawl.* Samarqandi wrote that *Galaba Burudat al-Mathana*and the muscles of uterus are weakens by the accumulation of *Fasid Mawad* (morbid matter).[25, 26] Further the mean age of patients was 40.91 years. According to Unani scholars, S*in al-Kahulah* ranges from forty to sixty years. In *Sin al-Kahulah,* the *Mizaj* is *Barid* and *Yabis*. Unani scholars were of opinion that in *Sin al-Kahulah* the *Mizaj* is toward *Burudat*. [10]

The strength of the present study was till date none of the studies, have evaluated *Mizaj* in women with MUI. Further, MUI was diagnosed based on MESA Questionnaire and for general *Mizaj* validated self-reported *Mizaj* questionnaire was used. Though current findings are important, the limitation of this study was test and re-test reliability of parameters used for assessments of *Mizaj* has been not carried out. Hence, further it is recommended to validate the*Mizaj* parameters in larger sample size, so that these parameters can be used for clinical assessment of different diseases.

Conclusion

This preliminary study validated the claim of Unani scholars that this disease is more common in *Barid Mizaj* and the *Su'-i-Mizaj* is towards *Burudat* in women with MUI. Thus, the above studies confirm the *Mizaj* theory in Mixed Urinary Incontinence.

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Conflict of Interest

The authors declare no conflict of interest.

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Physicochemical properties of two clay types (Termite mound clay and Potter wasp nests clay) that use in traditional medicine: A review

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Abstract

This review article contributes to clarify physical and chemical properties of termite mound clay (*Humbas meti*) and potter wasp nest clay (*Kumbal meti*) which are traditionally used medicaments in Sri Lanka. Clay types enriched with cations are widely used as major mineral ingredients in traditional pharmaceutical science of *Rasashastra* (alchemy), *Samhita grantha* and Ayurveda pharmacopeia. Pharmaceutical products containing these two clay types have been used for different kind of diseases in different types of prescriptions; specially in Charaka Samhita for the disease named *Urusthambha* and also hydrocele (*Mutraja vruddhi*) and various edemas (*Shotha*). The main objective of this literature review was to discuss about physical and chemical properties of these two soil types and special objective was elucidate whether there is a linking between these chemical properties and curing ability. For the data collection of this study the detailed literature review was done on the chemistry and scientific basis of termite mound clay, Potter wasp nest clay and pharmaceutical applications was carried out using published Ayurveda text books and research articles, available from Science Direct. Research articles showed that termite mounds are frequently enriched with soluble salts such as ammonium nitrate, exchangeable basic cations such as Ca^{2+}, Mg^{2+} and K^{+} and $CaCO_3$ compared to the adjacent topsoil. Spectroscopic data revealed the presence of SiO_2, and Al_2O_3 in appreciable quantities, while Fe_2O_3, CaO and MgO were in minor quantities in potter wasp nest clay. Infrared spectral analysis showed that nest clay samples are composite of quartz, feldspar and kaolinite. It is hard to find local research articles about physicochemical properties of these medicinally important clay types.

Keywords: Physicochemical properties, Potter wasp nest clay, Spectrophotometry, Termite mound clay

Introduction

Ethnopharmacological studies usually compacts with studies of various plant species and standardization and preparation of medicinal herbs. Additionally, some studies provide list of animals and their products that are useful in traditional medicine [1, 2]. Some articles have highlighted the medicinal use of animal products from the perception of historical literature, signifying the importance of these reports to traditional medicine [3, 4]. Few Literature have been recorded that the medicinal use of insects and derived products is very common in ancient practices[5, 6]. For an example honey and propolis, which products are derived from *Apis mellifera* are commonly used curative and preventive medicine. Nowadays scientific studies have confirmed its' antiseptic, anticancer and anti-HIV (Human immunodeficiency virus infection) effects [7, 8].

The use of medicinal clay in traditional medicine also goes back to ancient times. Indigenous peoples around the world are still using several types of clay that are products of some insects to cure diseases. Costa-Neto (2002) reported that bathing with the smoke from a burning nest of vespid wasp (*Protopolybia exigua*) prevents the "evil eye". The role of insect made clays in human health has experienced a revival in interest due to advances in modern instrumentation. According to literature termite mounds contain a wide range of minerals that are helpful for pregnant women, with high trace amounts of both iron and calcium. Aboriginal Pregnant women take termite mound soil 2 or 3 times per day during their pregnancies [9].

In Ayurveda medicine, termite mound clay and potter wasp nest clay have been used to cure hydrocele (*Mutraja vruddhi*), edema (*Shotha*) and rheumatoid arthritis (*Amavata*) (Ayurveda Aushada Sangrahaya) as well as for *Urusthambha* (spasticity of thigh muscles).

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The general objective of this study is to review the use of these two soil types in medicinal purposes and specific objective of this review is to elucidate whether there is a link between these chemical properties and curing ability.

Uses of potter wasp nest clay (*Kumbal meti*) and Termite mound clay (*Humbas meti*) in Ayurvedic Medicine

Ayurveda prescriptions (*Vattoru*) use termite mound clay and potter wasp nest clay to cure edema (*Shotha*) and hydrocele (*Mutraja vruddhi*). According to Ayurveda Pharmacopeia (Ayurveda Aushada Sangrahaya), claimed earth from a fireplace (*lipa mada pasin* in Sinhala) and potter wasp nest clay (*Kumbal meti*), grind with ash plantain juice and mix with Honey and then apply for hydrocele (*Muta vruddhi*). *Anethum graveolens*, Termite mound clay (*Humbas meti*), paddy expands and puffs up when heated mixed and grind with water for edema (*Shotha*). As well as Termite mound clay (*Humbas meti*), mustard powder (*Aba kudu* in Sinhala), ginger powder (*Inguru kudu* in Sinhala) grind with *Cinnamomum camphora* leaf juice *(Kapuru atthana kola* in Sinhala) and then boil and apply for rheumatoid edema (*Amavata*). One portion of Termite mound clay (*Humbas mati*) and one portion of ginger powder grind with hot water for edema (*Shotha*). In Ayurveda medicine, these clay types mix with herbal materials and thermal treatments have been done to increase curing ability or to remove the toxic factors [10]. Acharya Charaka has mentioned the therapy named as *Valmīka mṛttikādyutsādana* in Sanskrit language, and it is contained within the mud of ant-hill (termite mound clay/ *Humbas meti*), the root, fruits and barks of *Pongamia pinnata* (*Karanja*) and bricks should be made to a powder. This should be used for dry rubbing frequently for spasticity of the thigh muscles [11]. *Śyonākādi pariṣka pralepa* (paste) prescribed by Acharya Charaka for spasticity of the thigh as follows. Root of *Withania somenifera (Ashvagandha), Calotropis gigantean (Arka), Azadirachta indica* (*Neem*) or *Cedrus deodara (Devadaru),* any one of these drugs may be mixed with honey, *Brassica campestris* Linn (*Rakta sarshapa*) and mud of ant hill (termite mound clay/*Humbas meti*) before being used as thick paste as external preparation for dry rubbing or massage[12]. Drug represent in Ola leaf manuscript in Sri Lanka (Books series of *Talpate piliyam)* stated that the paste of *Sulpha* (*Gandaka*), *cinnamon* bark (*Kurundu pothu*) and termite mound clay obtain from the top of the ant hill better to cure different types of abscess[13]. In addition to that it has mentioned purified mercury *(Rasadiya),* fruit of *Cinnamomum camphora (Karpura),* worm casts (*Panu pas*), powder of bricks grind with juice of *Caryota urens (Sreetala)* used for disease called yaws[14]. As per Acharya Suśṛta application of paste of black mud of ant hill (termite mound clay/ *Humbas meti*) made with cow urine acts as an antidote for bite by bees and mosquito[15].

Reason for using termite mound soil by Aboriginal communities

Aboriginal people also use termite mounds for many different reasons. The main applications of termite mound soil are gastro intestinal disorders and related to pregnancy. Some other uses are treatment as abdominal or menstrual pains, mineral deficiencies, lactation and to cure wounds. Sometimes they use termitaria (Termite mound nests) for cooking and as mosquito repellent. Aboriginals use these mounds in several different ways. Many families have their own recipes. One way is break off small pieces of mound and then drop it directly in to the mouth. Some communities, hand size piece of mound is ground finely and mix with water, milk or tea and then drunk. Some aboriginals use termite mound clay externally. Mud baths are recommended for the treatment of rheumatism and arthritis. According to Dextreit (1976), clay can be used to cure iron deficiency, because it contains catalysts that work in infinitesimal doses to stimulate failing organs [16]. When clay is exposed to sun, air and rain, it becomes more active.

The number of elements such as calcium, iron, magnesium, potassium and sodium in termite mounds may be important in traditional medicine. The clay and in particular the kaolin fraction, may act as an absorbent anti-diarrhoeal and may help to alleviate digestive disorders. The high concentrations of elements in the mounds provide a potential nutrient source, mainly during pregnancy when the needs for elements such as iron are increased.

According to Foti (1994), there are three factors that have been identified in this study which could contribute to an explanation as to the Aboriginal preference for termitaria over soils, they are a general increase in concentration of selected elements, a higher concentration of 'bioavailable' elements and soluble iron and ionisable iron were present in most mounds whereas not detected in soils.

Methods of analyzing physicochemical properties of these two clay types

Most elements were determined by using atomic absorption spectrophotometry. A number of elements (Co, Cu, Fe, Mn and Zn) were directly diluted with deionized water if necessary. Some elements (Al, Ca, Mg, K and Na) required ionization agent to avoid interference. Dhembare (2013) used atomic absorption spectrometry to determine Ca, Mg, Cu, Mn, Zn and Fe whereas Potassium was determined by using flame photometry [17]. Total carbon content was determined by dry combustion using an Eltra CS500- apparatus. Total nitrogen (N) was determined by the Kjeldahl method. Mujinya *et al* (2013) used X-ray diffraction method to determine soil texture [18].

Physicochemical composition in termite mound clay

There was significant differentiation between physical and chemical properties of mound and adjacent soil. According to Dhembare (2013) when comparing with termite mound soil with adjacent soil the clay content of the termite mound was significantly higher than the surrounding soil (Table 1) pH of termite mound soil and surrounding soil was 7.17 and 7.67 respectively. Termites modified this pH up to 12.5 and Changes of mound pH depends according to the termite species and soil type. Electrical Conductivity of the mound soil was 0.29 dS/m in surrounding soil and 0.31 dS/m in mound soil.

Dhembare, 2013 showed that the test parameters such as organic carbon, phosphorus, K, Mg, Fe, Zn and Cu were inclining while N, Ca, S and Mn were decline in mound soil. This study highlights that termite mound soil properties are generally more than the surrounding.

Table 1: Soil properties of termite mound soil and surrounding soil

	Sand	**Clay**
Surrounding Soil	61.1%	29.5%
Mound Soil	38.9%	70.5%

X-ray diffraction reveals that the termite-mound materials are enriched in 2:1 clay, especially mica and expandable clay minerals. Selective dissolution analyses show that mound soil contain greater relative amounts of Manganese oxides and poorly crystalline Iron oxides, relative to the surrounding. Macro termite mounds are frequently enriched in soluble salts (e.g. ammonium nitrate), exchangeable basic cations (Ca^{2+}, Mg^{2+}, and K^{+}), and CaCO3 compared to the adjacent topsoil. According to Sarcinelli, et al, 2009 soil samples were collected from the walls and inner parts of termite mounds and also from adjacent soil. Chemical analyses showed that pH and the contents of organic C and N, P, Ca and Mg were significantly higher in termite mounds compared with adjacent areas [19].

Physicochemical composition in potter wasp nest clay

There are no any evidences of physical and chemical properties of potter wasp nest clay in Sri Lanka. According to foreign literature it was shown that there are significant differences in chemical composition of potter wasp nest clay and surrounding soil.

Wasps belong to order hymenoptera and suborder Apocrita. They make a mixture adhesively stronger and lighter than clay which is their source of building material. Clay soils are hydrous aluminum polysaccharides and it contains variable amount of iron magnesium, alkali metals and other cations. Wasps gather mud, moisten it and add their saliva which acts as cement to the mixture. Researchers said that saliva of the mud douber wasp contains phosphorus, Magnesium, Sulfur, Chlorine, Potassium, Calcium and unidentified elements.

According to Kamalu *et al* (2015) Calcium, Magnesium, Total iron, Chloride, Sulfate and phosphates are present significantly high amount in wasp nest clay. Potassium and Aluminum are slightly small amount when compare with adjacent soil [20].

Rodrigues et al., 2018 showed that ten major chemical elements were present in the wasp nests. They analyze that using fluorescence spectrometer. Silica is the most abundant element and major oxide that present, then aluminium oxide and iron oxide. Chemical elements were derived from mineral quarts (Silicon dioxide), laolinite ($Al_2Si_2O_5(OH)_5$), illite (K,H_3O) (AL, Mg, $Fe)_2$(Si, $Al)_4O_{10}((OH)_2,H_2O)$ and gibbsite $Al(OH)_3$.

Discussion

The role of clays in human health has experienced a revival in interest due to advances in modern instrumentation such as transmission electron microscopes (TEM), atomic force microscopy (AFM), and spectrophotometers that allow us to study surfaces of minerals within their natural environmental. Recent reviews regarding uses of clay in maintaining human health have focused on the ancient practice of eating

earth materials containing clay minerals, e. g. Aboriginal communities. Alternatively, clays have been used topically in mud spas to adsorb toxins from skin and provide heat to stimulate circulation for rheumatism treatment. Healing practices of ancient cultures may depend on clay minerals with powerful adsorptive and absorptive properties to treat a variety of tropical diseases. The high adsorption and absorption capacities, cation exchange capacity and extremely fine particle size of certain clays, e. g. kaolin group minerals are important reasons why these minerals are used to remove secretions, toxins, and contaminants from the skin. Thus, absorption or adsorption capacity may be the reason for using termite mound clay and potter wasp nest clay (which contain kaolin group minerals) for curing edema and hydrocele by Sri Lankan traditional practitioners.

Conclusion

It is unable to find research articles about the reason of using potter wasp nest clay and termite mound clay by Sri Lankan traditional physicians and Ayurvedic medical practitioners. Traditional physicians use these recipes on the basis of their indigenous knowledge and clinical practices in Sri Lanka. Occasionally they not aware of scientific theories behind these valuable medicines. Therefore, this is a better area to scientifically study the properties and medicinal value of these clay types.

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Mini review on *Mallotus phillipinensis* (Lam.) Muell. Arg. (*Kampillaka*)

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Abstract

Mallotus philippinensis (Lam.) Muell. Arg. (Family: Euphorbiaceae) is a medicinally important common perennial tree used in indigenous systems of medicine. It is distributed chiefly in the tropical and subtropical regions of the world. Though, it is a drug of herbal origin it has been categorized as one among eight *Sadharana rasa* in *Rasa-shastra* (Ayurveda study of minerals and metals) of Ayurveda pharmacopoeia. *M. philippinensis* is included in *Virecana ghana* of Ayurveda medicine. Specially roots, fruits and fruit powder and the leaves are used for medicinal purposes. Leaves are bitter, cooling and appetizer, the glands/hairs of the fruit and the leaves are recommended for dermal problems. Many scientific investigations have been carried out to validate and investigate the pharmacological activities of *M. philippinensis.* In the present study, an attempt was taken to summarize distribution, morphology, traditional uses and scientific investigations on *M. philippinensis.*

Keywords*:* Health benefits *Mallotus philippinensis* (Lam.) Muell. Arg., , Pharmacological activities

Introduction

Mallotus philippinensis (Lam.) Muell. Arg. (Family: Euphorbiaceae) is a large genus of trees and shrubs distributed chiefly in the tropical and subtropical regions of the world with around 20 species in India[1]. *Mallotus philippinensis* (*M. philippinensis*) commonly called in Sanskrit: *Kampilya, Kampillaka, Raktaphala, Recana*, in Sinhala: *Hamparila*, in English: *Kamala* and in Tamil *Kapila*[2]. Though, *M. philippinensis* is a drug of herbal origin it has been categorized as one among eight *Sadharana rasa* in *Rasa-shastra* (Ayurveda study of minerals and metals) of Ayurveda pharmacopoeia.

It is a common perennial tree, medicinally important plant used in indigenous systems of medicine. Trees are small to medium-sized monoecious in nature, up to 25 m tall and with a bole up to 50cm in diameter, but usually much less in number. Leaves are alternate and simple, more or less leathery, ovate to lanceolate, cuneate to round with two glands at base. Leaves are mostly acute or acuminate at apex, conspicuously 3-nerved, hairy and reddish glandular beneath, petiole size 1–4cm long, puberulous and reddish-brown in color. Male flowers in terminal and axillary position, 2–10cm long, solitary or fascicled paniculates spikes, each flower is with numerous stamens, small; female flowers have spikes or slender racemes, each flower with a stellate hairy, 3 celled ovaries with 3 papillose stigmas. Fruit is a depressed-globose; 3-lobed capsule; 5, 7 mm, and 10 mm; stellate; puberulous; with abundant orange or reddish glandular granules; 3-seeded. Seeds are subglobose and black in color and 4 mm across[3]. Mature fruits have glandular hairs and they collected as reddish brown powder. Specially roots, fruits and fruit powder and the leaves are used for medicinal purposes. Leaves are bitter, cooling and appetizer. Although, the glands/hairs of the fruit is the common use part of this herb for medicinal preparations and this pure red powder is not freely available in the market.

Pharmaco-dynamic properties of *M. philippinensis* are, *Rasa: Katu, Amla, Guna: Ushna, Ruksha and Teekshna, Virya: Ushna, Vipaka: Katu, Dosha Karma: Kapha Vata shamaka, Karma: Rechaka, Krimighna, Vibhedi, Ama - pachana, Deepana* and *Asrajit. Rogagnata: Krimi, Twak roga, Vruna, Vibandha, Gulma, Udara, Arshas, Shula, Jvara, Prameha and Prabhava: Recaka. M. philippinensis*, an *Audbhida dravya* is well described in Charaka and Sushruta samhita-Ayurvedic classics of ancient traditional medicine of India but, it was not much used in recipes of traditional medical system of Sri Lanka respectively.

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M. philippinensis is including in *Virecana ghana* of Ayurveda medicine.

Different preparations such as powders- *Kampillaka churna* for *Gulma, Udavarta, Krimi*, *Patolamuladi churna* for *Udara, Vatika-Krimighatani vati* for *Krimiroga, Malahara* -"*Kajjali Kodaya Malahara*" for wound cleansing, healing and chronic wounds for quick healing activity, *Varti- Krimignadi varti*, and oils –*Vipadikahara grita taila* are some examples which were mentioned in Ayurveda classics. Availability of these prepared medicines is less in market in Sri Lanka.

Health benefits of *Mallotus philippinensis*

Some traditional uses of this plant were mentioned in Ayurvedic classics. The glands/hairs of the fruit and the leaves of *M. philippensis* are recommended for dermal problems and the oil prepared with fruit powder and the gingili oil is better for wound healing[4]. Powder of *M. philippinensis* (glands/ hairs of the fruit) properly mixed with coconut oil can be applied for *Pama kushtha* (skin lesion with white and red, black, itchy eruptions), burning wounds and other wounds also[5]. *M. philippensis* is used to dress wounds, burns and the oil of *M. philippensis* cleanses chronic infected wounds. In dermatitis, especially of the oozing type, is considered to be a valuable remedy[6]. In addition, tincture of *M. philippensis* is prescribed for worm treatments as it contains purgative properties other than the wormicidal activity[2]. The dose of 1.5 g - 2g of fruit powder with *Guda* (Jaggery of sugarcane) is better for intestinal worms[2]. Along with this medicinal importance, this plant is used against human pathogens including *Helicobactor pylori*, anti-inflammatory activity, antiallergic, anti-HIV activity, and many more[3].When the fruit powder is mixed with *Shatadautagrita (ghee prepared by 100 times washing)* is better for hair falling. Also *M. philippensis* contains blood purifying and aphrodisiac properties[5]. *M. philippinensis* fruit is purgative, detergent, carminative, alexiteric and useful in treatment of bronchitis, abdominal diseases, spleen enlargement etc and if taken with milk or curd (yoghurt), it can be quite useful for expelling tapeworms [7]. The crude powder of kamala obtained as a glandular pubescence from the exterior of fruits is found to be useful in case of worm, hook worms, round and earth worms, anthelmintic activity. The drug was found to be 100% effective against tape worms[8].

Fruit powder of *M. philippinensis* is one of the main ingredients of *Vipadikahara grita taila* is a medicated oil which was mentioned in Caraka Samhita under the *Kushta chikitsa* as a treatment for five types of skin diseases-*Vipadika, Carma kushta, Eka kushta, Kitibha* and *Alaska*[9]. Scientifically, the efficacy of *Vipadikahara grita taila* against *Vipadika*- a skin disease with fissures of palms and feet with severe pain, was proven by Hewageegana and co-workers[10]. Further, *Vipadikahara grita taila* showed potent antibacterial activity against *Streptococcus pyogenes*, by agar well diffusion method by measuring the zone of inhibition[11].

Following are some positive scientific aspects which *M. philippinensis* is taken as a drug itself.

Antimicrobial Activity

The antimicrobial activity of hexane, chloroform and ethanol leaf extract showed significant activity against the human pathogens such as *Streptococcus pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi*, *Vibrio* species and the fungus *Candida albicans*. The antimicrobial activity of the tested extracts showed dose dependent activity against all the tested bacteria with the zone of inhibition ranged from 12-26 mm. However, only the ethanol extract showed antimicrobial activity against the tested fungus *Candida albicans* and with the zone of inhibition ranged from 16-22 mm[12]. Antimicrobial activity of hexane, chloroform and methanol extracts of stem bark of *M. philippinensis* was investigated against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and *Bacillus subtilis*. Except hexane extract, other extracts showed significant antimicrobial activity against tested bacterial spp.[13]. According to Zaidi and co-workers (2009)[14], 70% ethanolic extract and its constituents of *M. philippinensis* (at the concentration of 15.6–31.2mg/L) showed potent antimicrobial activity against *Helicobacter pylori*. Further, purification of extract revealed that rattler in exhibits potent bactericidal effect with minimal bactericidal concentration (MBC) of 3.12– 6.25mg/L especially against clarithromycin and metronidazole resistant *H. pylori* strains to prevent further surge in resistant antibiotics.

Hepatoprotective activity

Hepatoprotective activity of the methanolic extract was studied against various hepatotoxicants such as ethanol and carbon tetrachloride in rats. Physical (wet liver weight and volume), biochemical (Serum Glutamic Oxaloacetic Transaminase, Glutamic Pyruvic Transaminase, Alkaline phospate, direct and total bilirubin, total protein, cholesterol, triglyceride), Antioxidant Parameters (Catalase, Superoxide Dismutase, Lipid Peroxidation), Functional (thiopentone induced sleeping time) and histopathological changes of livers were assessed in control/standard/ and extract treated animals exposed to ethanol and carbon tetra chloride hepatotoxicants in rats. When compared to ethanol and carbon tetra chloride toxicant groups the increased thiopentone sleeping time, wet liver weight and wet liver volume was markedly reduced in methanolic extract treated groups. The toxicants induced a rise in the plasma enzyme levels of Serum glutamate oxaloacetic transaminase, Serum glutamate pyruvic transaminase, Total cholesterol, Total bilirubin, direct bilirubin, Triglycerides, Alkaline phosphatase and Thiobarbituri acid reactive substance (TBARS) like Malonaldehyde. This increase in the enzyme levels were significantly lowered by the extract at 100 mg/kg and 200mg/kg. Total protein was found to be decreased compared to normal control group. The toxicant decreased Catalase and SOD activities of liver. These activities were significantly increased by the extract of 100 mg/kg and 200mg/kg. The histo-pathological changes i.e. fatty changes (steatosis), necrosis etc were partly or fully prevented in animals treated with the extract[15].

Anti-Leukemic Activity

Root extract of *M. philippinensis* was tested on human promyelocytic leukemia HL-60 cell proliferation, cell cycle regulators, and apoptosis in order to investigate its antileukemic effect. Hexane fraction showed promising toxicity against p53-deficient HL-60 cells (IC50 1.5 mg dry roots equivalent/mL medium) after 72 h and, interestingly, inhibition of cell proliferation was preceded by the upregulation of the protooncogenes Cdc25A and cyclin D1 within 24 hours suggesting its antileukemic effect in HL-60 cells[16].

Anti-HIV Activity

Four phloroglucinol derivatives isolated from *M. philippinensis* were tested for their ability to inhibit the activity of human immunodeficiency virus- (HIV-) reverse transcriptase. The mode of inhibition of mallotojaponin was found to be competitive with respect to the template primer, (rA)n (dT)12–18, and noncompetitive with respect to the triphosphate substrate, dTTP. The Ki value of mallotojaponin for HIV-reverse transcriptase was determined to be 6.1 μM[17].

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using ethanol (50%) extract of glandular hairs of *M. philippinensis* fruits in Charles-Foster albino rats. Three animal experimental models were used: (a) carrageenan (acute) (b) turpentine oil induced formalin (sub-acute) induced paw edema (c) granuloma pouch (sub-acute). *M. philippinensis* at a dose of 200 mg/kg at 3 h after their administration showed inhibition of formalin-induced paw edema by 41.60% and carrageenan-induced paw edema by 55.30%. After 7 days of treatments, *M. philippinensis* showed 38.0% inhibition against formalin-induced paw edema and reduced weight of turpentine induced granuloma pouch by 29.6% and volume of exudates by 26.1% respectively[18]. Daikonya and co-workers[19] have shown inhibition of nitric oxide (NO) production and inducible NO synthase (iNOS) gene expression by a murine macrophage-like cell line (RAW264.7) which was activated by lipopolysaccharide (LPS) and recombinant mouse interferon-gamma (IFN-gamma) using the hexane fraction of acetone extract of *M. philippinensis* fruits. In addition, suggest the down regulation of cyclooxygenase-2 gene, interleukin-6 gene, and interleukin-1b gene expression.

Analgesic and hypnotic activity

Analgesic activity was evaluated using ethanol (50%) extract of glandular hairs of *M. philippinensis* fruits in Charles-Foster albino rats. Three animal experimental models were used: tail-flick, hot-plate, and acetic acid-induced writhing tests. Results revealed that *M. philippinensis* at a dose of 200 mg/kg, showed dose-dependent elevation in pain threshold and peak analgesic effect at 120 min as evidenced by increased latency period in tail flick method and increased reaction time in the hot-plate test while the reduction in the number of acetic acid-induced writhes by

45.7%. Hypnotic activity was investigated by pentobarbitone-induced hypnotic potentiation in rats and sleeping duration was significantly prolonged in rats treated with *M. philippinensis* at a dose of 200 mg/kg[18].

Antioxidant activity

Extracts of *M. philippensis* fruits and bark were evaluated for total antioxidant activity, DPPH (2,2-diphenyl-1- picrylhydrazyl radical) scavenging activity, reducing power, total phenolics and tannin contents. The extract of the bark showed the strongest antiradical activity and reduction power[20, 21].

Conclusions

Present review confirms the medicinal values of *Mallotus philippensis* and it can be used against human pathogens and a promising candidate for hepatoprotection, anti-leukaemic, anti-HIV, anti-inflammatory, analgesic, hypnotic, antioxidant potential and healing skin lesions. These findings may lead to further development of novel pharmaceutical preparations from *M. philippensis* in the future.

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Chronic anti-inflammatory effect of bees' honey on Freund's Complete Adjuvant induced arthritis in rats

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Abstract

Bees' honey has been used to treat in many diseases in traditional medicine. The usage of bees' honey as a medicine is referred to in the most ancient Ayurveda medical records. Therefore, this study was design to evaluated the chronic anti-inflammatory effect of bees' honey using adjuvant-induced arthritis in experimental rat model.

Healthy male Wistar rats were randomly assigned into 4 groups (n=6 in each). Arthritis was induced by a single intra-dermal injection of Freund's Complete Adjuvant (FCA) containing *Mycobacterium butyricum* suspension into a foot pad of the left hind paw of all groups of Wistar rats except healthy control group (Group I). Group II - arthritic rats were received distilled water. Group III-arthritic animals treated with a standard drug Celecoxib (5mg/kg) and Group IV-arthritic animals were received BH (4ml/kg). Following induction of arthritis, daily oral treatment was started on day 14 and continued up to day 28. Body weight, ankle joint thickness and foot pad thickness were measured in all animals using dial calliper on Day 0 and on Day 3,7,10,14,17,21,24 and 28 after the injection of adjuvant. Full blood count was tested on day 28. Induction of arthritis significantly increased FPT, AJT and loss of BW. Treatment with Bees' honey and standard drug Celecoxib in the arthritic animals produced significant reductions respectively $p<0.05$, $p<0.001$ in FPT, AJT, WBC count, reducing erythema and oedema in the ankle joints and foot pad of the AIA rats and normalized BW. This study provided transitional medicinal evidence for BH used in the treatment of chronic inflammation in traditional medicine.

Keywords: Arthritis, Bees' honey, Chronic anti-inflammatory effect

Introduction

For centuries, Bees' honey (BH) has been used as effective and well-deserved reputation healing agent for various diseases in different traditional systems of medicine. Vitamins and trace elements contained in honey have a positive impact on all the vital processes of the human body. BH was prescribed by the physicians of many ancient races of people for a wide variety of ailments.

According to the Charaka Samhita, honey is of four types; *Makshika, Bhramara, Kshaudra* and *Paittaka. Makshika*, the best type of honey is produced by reddish variety of honey bee. This type of honey is the color of *Tila taila* (sesame oil). *Bhramara* honey is produced by the *Bhramara* type of bee. It is Guru (heavy) and is of white color. *Kshaudra* honey is produced by a small type of honey bee and is brown in color. *Paittaka* honey is produced by a large type of bee and is of the colour of ghee[1]. According to Susrutha Samhita, honey is of eight types[2].They are *Makshika* (Bees' honey), *Bhamara* (honey produced by Bumble) , *Argya* (honey produced by Wasp), *Pouthika* (honey produced by tiny insect call *Kannei*), *Ouddhalaka* (honey collected in anthill), *Kshaudra* (honey produced by species of tiny bees), *Dala* (honey collected in flower petals), *Chatra* (honey collected by a certain kind of bees whose hive like an umbrella).

Out of these eight honeys, the variety produced by honey bees is the most commonly referred type of honey. BH is recommended as an *Anupana* (vehicle) for in pediatrics age group in Ayurveda[3, 4]. According to the Ayurvedic authentic texts, BH was widely used in the treatment of ophthalmic disorders, jaundice, piles, tuberculosis, asthmatic conditions and, respiratory disorders. Old bees' honey helps to reduce over weight. Literature survey revealed that BH was most useful as an *Anupana* (vehicle*)* in Ayurvadic medicine against inflammation but no scientific evidence is available for its chronic anti-inflammatory potential.

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Thus, this present study was focused to evaluate the chronic anti-inflammatory effect of BH in experimental rat model.

MATERIALS AND METHODS

Bees' honey

Fresh BH was collected from Millaniya division in Kalutara and authentication was conducted with standard BH by chromatographically at the Department of *Dravyaguna Vignana*, Institute of Indigenous Medicine, University of Colombo.

Animals

Healthy, unused male Wistar rats (200-250g) were purchased from Medical Research Institute (MRI), Colombo, Sri Lanka. The animals were kept in plastic cages (two per cage) under standardized animal house conditions (temperature, 28–31°C; photoperiod, approximately 12 h natural light "per day" relative humidity, 50–55%) at the animal house of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka with access to food and water ad *libitum*. A period of one week was given for acclimatization to animal house conditions prior to the commencement of the experiments.

Animals were euthanized under anesthesia following completion of the experiment with an overdose of aesthetic ether. All animals were humanely treated in accordance with WHO and Federation of European Laboratory Animal Science Associations (FELASA) guidelines for animal care.

All experiments in rats were carried out in accordance with the recommendation of the guidelines for care and use of laboratory animals and the project proposal was approved (No.591/11) by the Ethics Review Committee of the Faculty of Medical Sciences of the University of Sri Jayewardenepura, Sri Lanka.

Chronic anti-inflammatory effect

Chronic anti-inflammatory activity of BH was analyzed using adjuvant-induced arthritis rats model[5]**.** Arthritis was induced by a single intra-dermal injection of 0.1 ml of Freund's Complete Adjuvant (FCA) containing 0.05% w/v *Mycobacterium butyricum* suspension in sterile paraffin oil into a foot pad of the left hind paw of all rats with help of glass syringe and 26 G needles after the rats were subjected to light diethyl ether anesthesia[6]. The healthy control group was injected with a single dose of 0.1 ml of normal saline into a foot pad. The animals were randomly divided to four groups of 6 rats each (Table 1).

Drug administration

On the 14th day, each group was treated with medication by intragastric administration once daily for next 14 days[7]. All the rats were orally treated for 14 consecutive days from the day 14 to till 28th day.

Body weight examination

Body weight (BW) of the rats was measured on day 0, 4, 8, 12, 16 and 20, 24, and 28 after induced. The body weight gain of each animal was calculated using the following formula[8].

$$\text{Body weight gain} = \text{Body weight on day N} - \text{Body weight on day 0}$$

Measurement of ankle joint thickness (AJT) and foot pad thickness (FPT) of the rats

The hind paw ankle joint thickness (AJT) and Foot pad thickness (FPT) of all animals were estimated on day 0 (before injection of FCA emulsion) and on day 3, 7, 10, 14, 17, 21, 24 and 28 after the injection of adjuvant. Dial Caliper (Mitutoya, Japan) was used for measuring the AJT and FAT[9].

Percentage inhibition of change in ankle joint thickness and food pad thickness was calculated by using the following formulas;

$$\text{AJT (\%)} = \frac{(\text{Treated group}_{AJT} - \text{Healthy control}_{AJT})}{\text{Healthy control group}_{AJ}} \times 100$$

$$\text{FPT (\%)} = \frac{(\text{Treated group}_{FPT} - \text{Healthy control}_{FPT})}{\text{Healthy control group FPT}} \times 100$$

Sample collection

All animals were sacrificed on day 29th and blood was collected in EDTA-coated vials: for Full blood count (FBC) by automated haematology analyzer (Dirui BCC-3000 B, China).

Table 1: Treatment groups and the drugs the rats were given

	Group Name	Drug
Non arthritic rats	Healthy control	Distilled water (2.5 ml)
Adjuvant Induced Arthritis rats (AIA)	Arthritic control	Distilled water (2.5 ml)
	Standard	Celecoxib (5 mg / kg)
	BH	Bees' honey (4ml/kg)

Statistical analysis

The data were expressed as arithmetic mean ± SEM. Untreated arthritic rat group was compared with healthy control animals and the treated arthritic groups were compared with untreated arthritic animals. The significance level was determined using student's t-test and considered extremely significant (***) $p<0.001$; highly significant (**) $p<0.01$; significant (*) $p<0.05$; and not significant ($p>0.05$).

Results

Body weight of rats

Following FCA emulsion injection, there was no significant changes in the body weight as observed on Day 0 and 7 in arthritic rats. The body weight of negative control rats was significantly ($p<0.001$) compared with healthy control animals. But arthritic rats treated with standard drug Celecoxib and BH showed significant weight gain after day 14, as compared to negative control animals. After 14 days, weight gain of rats was gradually increased in treated groups (Figure 1).

Effect on ankle joint thickness (AJT)

At Day 0, no significant differences were found among the AJT of all studied groups. A significantly enhanced in AJT was found in the FCA injected group of animals on Day 3 (first swelling phase). Thereafter, swelling slowly subsided until seventh day and then began to increase again when disseminated arthritis appeared. After the initiation of drug administration on Day 14, significant reductions of AJT in AIA group of animals on Day 17, 21, 24 and 28 were observed as compared to the arthritic control rats. The test groups Bees' honey exhibited significant ($p < 0.05$) reductions of the AJT. Celecoxib showed extremely significant ($p < 0.001$) reductions of AJT on Day 28 as compared to the arthritic control rats. The rats treated with BH have also shown ($p<0.05$) significant reduction of AJT following the oral administration (Figure 2).

Effect of foot pad thickness

On Day 0 beginning of the experiment, no significant differences were found in the rat FPT among all the groups. A significant increase in FPT was observed for the adjuvant injected group on Day 3. Swelling and redness developed over a 24-h period in the hind paw injected with CFA and reached maximum intensity on day 3 (first swelling phase). Thereafter, swelling slowly subsided until the seventh day and then the FPT began to increase again when disseminated arthritis appeared (second swelling phase, which was greater than the first one and peaked on days 21–24). Foot pad thickness of AIA rats were observed on Day 7, 10, 14, 17, 21, 24 and 28 as compared to the healthy control rats and showed statistically reduction ($p < 0.001$). There were no significant differences ($p> 0.05$) between AIA rats on Day 4, 7 and Day 14.

After 14^{th} day, treatments were started with the standard drug celecoxib and BH. Significantly reduced of foot pad thicknesses were observed on Day 17, 21, 24 and 28. Extremely significant effects ($p < 0.001$) were observed in celecoxib group on Day 21, 24, and 28; whereas in significant differences of FPT were observed after 14^{th} day BH group as well. The reduction of foot pad thickness taken as a marker of disease recovery in AIA rats is shown in Figure 3.

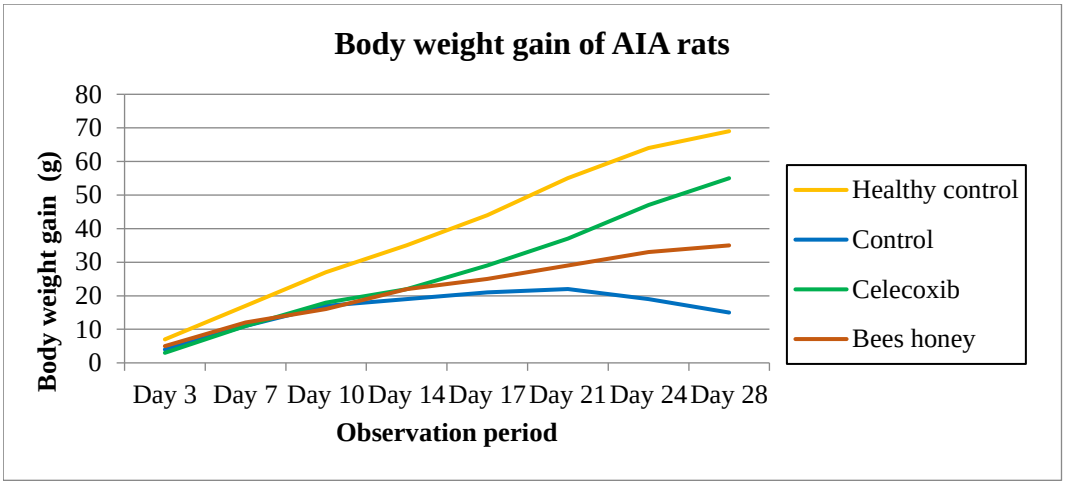

Figure 1: Effect of drugs on body weight gain of AIA.
The data are expressed as mean ± SEM (n=6 per group). $p<0.05$; and not significant ($p>0.05$)

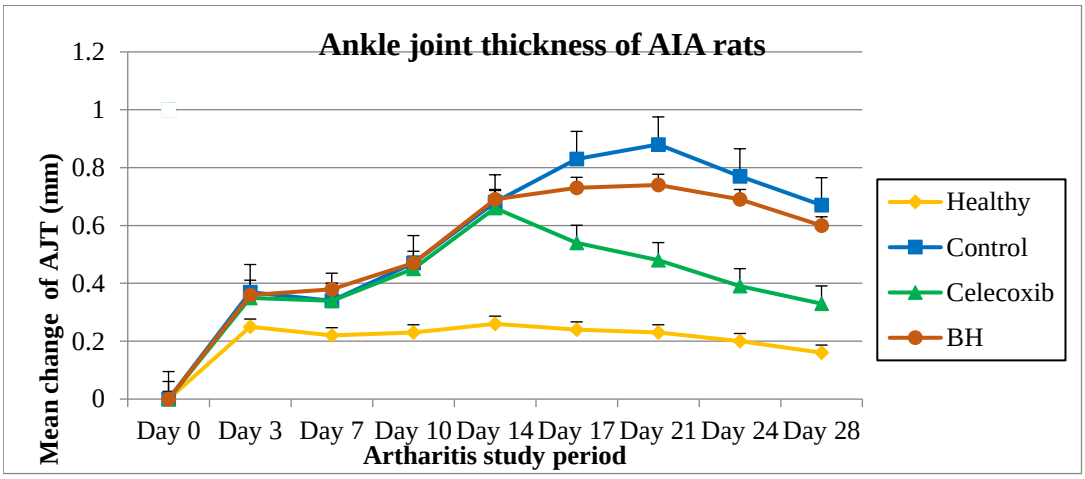

Figure 2: Effect of drugs on ankle joint thickness (AJT)

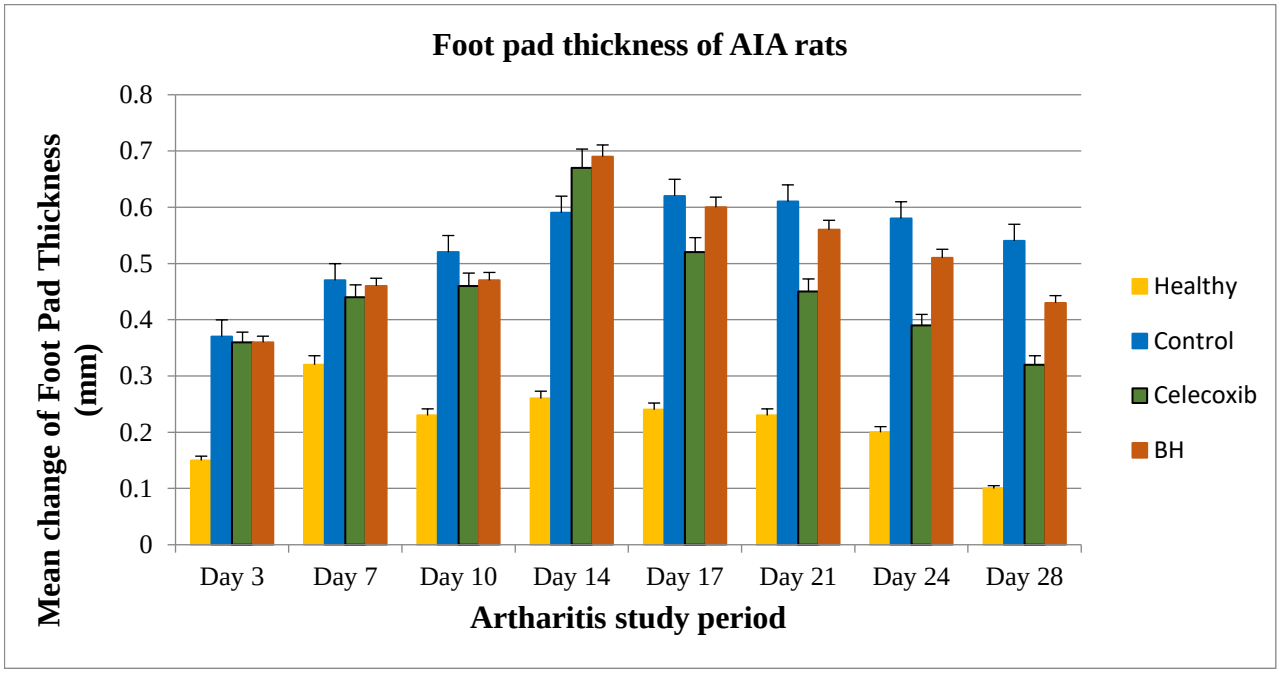

Figure 3: Effect of foot pad thickness in adjuvant-induced arthritis model

Table 2: Effect of drugs treatment on haematological indices in arthritic rats

Blood parameters	Groups			
	Healthy	Control	Celecoxib	BH
White blood cells (WBC) (109/L)	8.2 ± 0.2	18.2 ± 0.2	10.6 ± 0.3***	16.2±0.5*
Neutrophil (%)	31.9 ± 3.6	13.1± 1.4	22.3 ± 0.9***	16.1 ± 2.9
Lymphocytes (%)	55.8 ± 2.0	81.2 ± 1.3	71.1 ± 0.8***	75.4 ± 2.1*
Monocyte (%)	4.9 ± 1.7	5.8 ± 0.8	6,6 ± 0.6	8.4±0.9
Red blood cells (RBC), (109/L)	8.1 ± 0.5	6.3 ± 0.4	8.3 ± 0.5**	6.95 ± 0.3
Hemoglobin (Hb) (g/dl)	14.9 ± 0.3	13.3 ± 0.3	14.2 ± 0.3	13.5 ± 0.2
Packed cell volume (PCV) (%)	51.3 ± 2.3	31.7± 3.0	37.7 ± 1.8	33.7± 3.1
Platelets (109/L)	836.6 ± 3.5	832.6 ±9.2	866.6 ± 11.0*	852.6 ± 11.8

The data are expressed as mean ± SEM (n=6 per group). Symbols represent statistical significance: extremely significant (***) $p<0.001$; highly significant (**) $p<0.01$; significant (*) $p<0.05$; and not significant ($p>0.05$).

Effect of drugs on haematological indices of AIA rats

There was a significant ($p<0.01$) decrease observed in Heamoglobin (Hb) and Red blood cells (RBC) concentrations in AIA control group, when compared to the healthy control rats. Moreover, white blood Cells (WBC), neutrophils and lymphocytes also showed significant differences when compared to the healthy control group.

In the AIA rats treated with Celecoxib was significant ($p < 0.001$) reduction in the WBC and lymphocytes and significant ($p < 0.01$) increase in RBC (Table 2). In the group given Celecoxib, there was a significant ($p<0.001$) increase in PLT when compared with the AIA control group. Bees' honey had the capacity to decrease WBC and lymphocytes ($p <0.05$).

Discussion

Adjuvant-induced arthritis (AIA) in rats is a useful tool to study the pathophysiology of Rheumatoid Arthritis (RA), especially because the experimental model and the human disease share various signs and symptoms[10]. Therefore, in the present study an intra-dermal injection of FCA containing heat-killed cells of *Mycobacterium butyricum* was used to induce inflammation and arthritic lesions in the animals.

Anti-arthritic potency of drugs was determined by reversal of the altered arthritic parameters. The result of Figure 3 shows that the foot pad thickness (FPT) increased in adjuvant- challenged animals. Drug administration suppressed severity of clinical arthritis, as demonstrated by decreased FPT in rats.

Previous report showed that there was significant body weight loss, the day following injection of the adjuvant[11]. The result of the present study also indicates that there is close relationship between the extent of inflammation and loss of BW. The body weight of negative control rats was significantly decreased compared with healthy control rats. The data suggested that oral treatment of Celecoxib and BH recovered inflammatory body weight loss in arthritic animals. The body weight of the rats treated with BH significantly ($p<0.01$) increased compared with negative control animals after starting the oral treatment.

Similarly, body weight in arthritic animals was enhanced by Celecoxib as well as BH administration as shown in Figure 1. The observed changes in AJT of the experimental groups of animals are shown in Figure 2.

The percentage of AJT was significantly enhanced in the negative control group of arthritic animals as compared to healthy control rats. Recovery from increased AJT in adjuvant injected rats, BH treated animals showed better results in certain extent similar to that of standard drug Celecoxib.

Adjuvant-induced arthritis study was carried out to investigate the chronic anti-inflammatory activity of BH in rheumatoid arthritis rats. In this study, adjuvant injected paw was illustrated by a rapid onset of inflammation evident within 24 h of adjuvant injection and continued for 28 days.

Administration of FCA led to increase in the total blood leucocyte count suggesting the involvement of WBCs in response to antigen-mediated arthritic[12]. A significant increase in WBC count was observed in the AIA control rats when compared to the healthy control rats. However, there was a significant difference ($p<0.05$) of the WBC counts observed in the BH treated group when compared with the baseline values and this observation needs further investigation.

According to the findings of the present study Bees' honey has proven its potent chronic anti-inflammatory effect.

Conclusions

This study revealed that the promising chronic anti-inflammatory effect of BH in rats which was reported scientifically for the first time. As found in the present study, bees' honey exerts chronic anti-inflammatory effects that are closely similar to standard drug Celecoxib. This study provided transitional medicinal evidence for BH used in the treatment of chronic inflammation in traditional medicine.

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