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Cover story

Wel Bakmi

***Nauclea orientalis L.* (=*Sarcocephalus cordatus* (Roxb.) Miq.)**
Family: RUBIACEAE
Vernacular names: Sinhala: *Bakini, Bakmi, Rata-bakmi:* **Sanskrit:** *Kadamba;* **English:** *Yellow Cheese wood*[1]*;* **Tamil:** *Wellai Kadambu, Athuvangi*[2]*, Vammi*

Nauclea is a genus of flowering plants (Angiosperm) native to Bangladesh, Philippines, Sri Lanka, Thailand, and South East Asian countries. There are around 13 Nauclea species of evergreen trees or shrubs in the family Rubiaceae, including *Nauclea orientalis*, which is a small tree with a height of 45m, trunk diameter of 100-160 cm, a broad crown and a straight cylindrical bole.

The bark is gray, smooth in young trees and rough, longitudinally fissured in old. It has glossy green, opposite, ovate to elliptical leaves and bisexual, fragrant, orange/ yellow flowers. *Kadamba* is closely allied to the subtribe Naucleinae (Rubiaceae) but differs in its placentation mode. The species is in the focus of a classification controversy based on the name of the original type specimen described by Lamarck[3].

It is a tree of the moist tropical lowlands found at elevations from sea level to 500 meters, where it is best grown in medium and clay loams with 30 - 40°c annual daytime temperature and 1,000 - 3,500mm mean annual rainfall. It always prefers semi-shaded conditions and succeeds in most soils, though it prefers alluvial soils along stream banks.

According to Ayurveda, Bakmi is dominant in Pungent, Bitter and Astringent taste, Cold potency and *Tridosha* alleviating action[4]. Its bark, leaves, roots and juice of fruits have been used in various forms for ulcers, conjunctivitis, mouth ulcers, vomiting, diarrhea, urinary calculi, and jaundice since Vedic period in India and a time unmemorable in Sri Lankan *Deshiya Chikitsa.*

Kabamba is an ingredient of *Vedana-sthapana* and *Shukra-shodhana Ghana* in Charaka Samhita[5] and *Nyagrodadi* and *Lodradi ghana* in Susruta Samhita[6]. Its leaves and bark are used against abdominal pain, animal bites, and wounds. The leaves are applied externally to boils and tumours. Studies have shown that the bark has moderate in vitro activity against the malaria-causing *Plasmodium falcifarum* and four new alkaloids; Nauclealines A and B and Naucleosides A and B, together with six known compounds were isolated.

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Contents

An Evidence Based Novel Treatment Protocol in the Management of *Peenasa* (Allergic Rhinitis) - A Case Report

Narathota S.N.L.[1*], Jayasiri A.P.A.[2] and De Silva L.D.R.[1]

Abstract

Peenasa characterized with rhinorrhoea, sneezing, watery nasal discharges and nasal blockage which hinder the health-related quality of life, can be compared with allergic rhinitis due the similarities of clinical manifestation. Allergic rhinitis is an immune reaction of nasal mucosa to allergens like pollen and dust which affects between 10% and 30 % of the population globally. Ayurveda classify *Peenasa* under *Nasagatha roga* (diseases pertaining to nose) while Traditional system of medicine considered it as a *Sarvanga roga* (disease pertaining to whole body). Present study was aimed to find the effectiveness of a novel Ayurveda treatment protocol in the management of *Peenasa*. A 19 years old male patient presented with frequent sneezing, watery nasal discharges, sore-throat and nasal congestion specially in the morning for a time period of two years. Based on the clinical examination, patient was diagnosed with *Peenasa* and was treated internally with *Triphala kwatha*, *Triphala churna* and externally with *Rathulunu thailaya* (RT) as *Shiro abhyanga* (head application) and *Prathimarsha nasya* (errhine therapy) for a period of two months. Evaluated the effectiveness of the novel treatment line on clinical features before and after the treatment. Patient showed significant reduction of clinical features of *Peenasa* within a short period of time. Ingredients of the prescribed drugs were prominent with *Katu rasa* (pungent taste) and *Ushna veerya* (hot potency) which helps to mitigate the pathogenesis of phlegmatic conditions. Further, anti-inflammatory, anti-oxidant, anti-allergy properties of the ingredients play a major role in pacification of vitiated *Dosha*. Thus, the novel treatment protocol can be considered as safe and effective in the management of *Peenasa* (Allergic rhinitis).

Keywords: *Peenasa*, Allergic rhinitis, *Triphala*, *Rathulunu thailaya*, Anti-inflammatory

Introduction

Ayurveda with its holistic approach offers a complete system to live a long life by maintaining healthiness and rejuvenating the body through diet and herbal medicines[1]. Maintaining good health by balancing physical, mental and spiritual aspects are highlighted in order to treat and prevent from diseases. Nowadays much more attention has directed towards treating diseases with this valuable medical system throughout the world. Well prepared, quality herbal drugs following chemical analysis and clinical trials help to add scientific validity and value to this holistic medical system.

According to Ayurveda and Traditional system of medicine in Sri Lanka, *Peenasa* is a phlegmatic disease caused mainly due to vitiation of *Kapha* and *Vata dosha*[2], which can be correlated with Allergic rhinitis due to the similarities in clinical manifestation[3]. Both *Kapha* and *Vata dosha* get aggravated with cold as both these *dosha* are associated with cold property (*Sheetha guna*)[4]. Symptoms of Allergic rhinitis include sneezing, rhinorrhea, nasal congestion, anosmia, headache,

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itching of nose, eyes, ears, palate, postnasal drip, tearing, red eyes, drowsiness and malaise[5]. Nasal congestion, itching of nose, eyes, drowsiness, malaise caused with vitiation of *Kapha* due to heavy (*Guru*), slimy (*Mruthsana*) properties whereas symptoms like tearing, sneezing, anosmia and pain are due to vitiation of *Vata dosha.* Vitiated *Pitta* causes dryness of the nose, burning sensation and nasal obstruction in *Peenasa*[6]. By considering the clinical features of *Peenasa,* this can be compared with Allergic rhinitis which occurs due to allergic reactions to foods, hereditary causes, seasonal changes etc. It is one of the commonest respiratory tract disorders[7]. Susrutha Samhitha mention *Peenasa* among the 31 types of *Nasagatha roga* (diseases pertaining to nasal area) [8]. Traditional medical system classifies *Peenasa* as a *Sarvangagatha roga* (disease pertaining to the whole body)[9] and different classifications are documented. In traditional texts it is known as *Diya peenasa, Sotu peenasa* or *Sem peenasa*[9]. Ashtanga Hrda Samhitha[10] and Bhava Parakasha[11] mention that both *Peenasa* and *Apeenasa* are a same disease condition. Common signs and symptoms of *Peenasa* are *Nasa srava* (rhinorrhea), *Kshuth* (sneezing), *Anahyathe* (nasal blockage), *Vidhupyathe* (smoky sensation), *Shirah shula* (headache) and *Shirah gaurava* (heaviness of head)[8].

Worldwide, allergic rhinitis affects between 10% to 30 % of the population. Most people with allergic rhinitis have mild symptoms that can be easily and effectively treated[7]. Though there are many documented and practiced Ayurveda treatments available for *Peenasa,* lack of scientific evidence is a major problem in the health sector. Hence, it's a timely necessity to find effective treatment protocols for the management of *Peenasa* to improve the Ayurveda medicinal system. Thus, the present study was focused to observe the effectiveness of a novel Ayurveda treatment protocol in the management of *Peenasa* (Allergic rhinitis).

Materials and Methods

Case report

A nineteen (19) years old male patient visited the Ayurveda Teaching Hospital, Borella with the clinical features of frequent sneezing with watery nasal discharge, sore-throat, on and off headache and nasal congestion specially in the morning for nearly two (02) years. As a student he had experienced disturbance in his studies and he had got allopathic treatments time to time during past 2 years. According to the patient he had relief only when taking the prescribed tablets and the nasal spray. No any other complaints were recorded other than indigestion and flatulence time to time. Past history of Bronchial asthma (BA) in childhood was present and no any related family history of allergic rhinitis was reported. Home-made food was mainly consumed and the patient has not taken cool drinks as it increases his rhinitis condition. No any habits and addictions were reported. Patient had a sound sleep and sometimes evening bath was reported. There was no evidence of drug allergy, but had experienced itchy skin rash after intake of pineapple. Written consent was taken from the patient to follow the novel treatment protocol and for the publication of data without disclosing the identity.

Clinical examination

In *Ashtavidha pareeksha* (eightfold examination), it was observed that *Nadi* (pulse) was 72bpm and no any abnormalities in urination [frequency D/N- 6-7/1-2], bowel moments [frequency D/N- 1-2/0, No hard stools]. *Jihva* (tongue) was slightly coated (*Ama*), *Shabdha* (sound) persistent mouth breathing was observed, Wheezing was not present but slight rhonchi was noted in auscultation. *Sparsha* (touch), *Drik* (vision) and *Akruthi* (body structure) was normal. In *Dashavidha pareeksha* (tenfold examination), it was observed that patient's *Prakruthi* (constitution) was *Vata-kapha,* state of disease are frequent sneezing with watery nasal discharge, nasal congestion, sore-throat and headache. *Sara* (systemic strength), *Samhanana* (compactness), *Sathmya* (suitability), *Sathva* (mental status) was normal. *Ahara shakthi* (digestive capacity) was normal with

normal appetite and bowel moments without constipation or diarrhea. But history of indigestion was present time to time. *Bala* (strength) was moderate to the *Vaya* (age) and *Vyayama shakthi* (power of exercises) was normal.

Nasal and oral Examination

Nasal polyps were not observed in nasal examination, but reddish appearance of nasal mucosa was present. Bilateral swollen tonsils were observed in throat examination.

Diagnosis of the patient

Considering the patient's history and the clinical examination the disease was diagnosed as *Peenasa* (Allergic rhinitis).

Treatment protocol

Internal and external treatments given to the patient are listed in Table 1 and Table 2. Patient was asked to avoid food with cold potency as cucumber, watermelon, ice-cream, cool drinks, cool water, curd. Also advised to take warm water and to avoid bathing in evening/night, awakening at night etc.

Triphala can be administered using different drug preparation methods and in the present study, *Triphala kwatha* (preserved form of decoction) and *Triphala churna* (medicinal powder) (Figure 1) was administered internally in the treatment protocol. *Triphala kwatha* was given 15ml (01 table spoon) twice a day, before meal in morning and evening for four (04) weeks. *Triphala churna* was given 2.5g (1/2 tea spoon) twice a day after meal, morning and evening for six (06) weeks.

Rathulunu thailaya (RT) (Figure 2) was prescribed to apply on head twice a day for 2 months and *Prathimarsha nasya* using 2 drops of RT for each nostril with the dropper in morning in 2nd and 4th week of the treatment plan.

Follow up

After completion of the treatment protocol, the patient was followed for 08 weeks at intervals of 07 days. Patient was completely free from the previous signs and symptoms of the disease and new complaints were not reported during the follow up.

Results

Research was mainly carried out to assess the efficacy of the novel treatment protocol used to treat the patient suffering from *Peenasa* (Allergic rhinitis). Results were analyzed according to the clinical manifestation of *Peenasa* before and after the treatments, during the phases of follow up (Table 3). Pharmacological properties and chemical composition of ingredients of the drug formulae are mentioned in Table 4 and Table 5.

After following the treatment plan for two (02) months, patient was free from signs and symptoms. Other complaints like flatulence, indigestion had also reduced with the treatment.

Pharmacodynamic properties of used medicines according to Ayurveda

Triphala kwatha and Churna

In analysis of Ayurveda pharmacological properties of the ingredients, it was observed that *Triphala* was prominent with *Kashaya rasa* (astringent taste) [100%], *Laghu* (light) [67%], *Ruksha* (rough) [33%] properties, *Ushna veerya* (hot potency) [67%] and *Madhura vipaka* (sweet final digestive taste) [100%][13]. *Ushna veerya* is important in pacifying vitiated *Kapha dosha* and no *Prabhava* (special potency) was identified (Table 6).

Rathulunu thailaya

It was observed that eleven ingredients of *Rathulunu thailaya* was prominent with *Katu* (pungent), *Thiktha* (bitter), *Kashaya* (astringent) *rasa* (tastes), *Laghu* (light), *Theekshna* (sharp) *guna* (properties), *Ushna veerya* (hot potency) and *Katu vipaka* (pungent final digestive taste)[13].

All the specific properties of *Triphala* and *Rathulunu thailaya* are important in pacifying vitiated *Kapha*, *Vata* and *Pitta dosha* which are responsible in developing *Peenasa*.

Analysis on chemical composition of Triphala and Rathulunu thailaya (RT)

Ingredients of Triphala (*Kwatha* and *Churna*) and RT possess with anti-inflammatory, anti-infective, anti-microbial and anti-cancerous properties. Anti-oxidant activity is also present in most of the drug ingredients of RT and *Triphala*[14,15]. The major constituents of the *Triphala* are the tannins, gallic acid, ellagic acid and chebulinic acid, which are potent antioxidants that may account, at least in part, for the observed immunomodulatory activity of the formula. Tannins are medicinally significant due to the astringent property which promote rapid healing and the formation of new tissues on wounds and inflamed mucosa[15]. Eugenol which is also known as clove oil is present in RT and used topically to treat toothache and more rarely to be taken orally to treat gastrointestinal and respiratory complaints[16]. Eugenol revealed pharmacological properties such as anesthetic and analgesic effects, antimicrobial, anti-oxidant, anti-inflammatory, anticarcinogenic and anti-fumigant activities[17]. Myristicin is common in nutmeg and is an insecticide and has potent anti-cancer properties[18]. Elemicin which is common in Nutmeg also present with anti-inflammatory properties. Zingiberene is effective against sore throat, helminthic and infectious diseases[19] which has medical properties including anti-nausea, anti-inflammation, anti-pyrexia and analgesia in addition to culinary uses. Linoleic acid [20] is one of two essential fatty acids for humans which must obtain through diet. Sesame seed is rich with unsaturated fatty acids, mainly linoleic acid (37-47%) and helps to reduce inflammation[21]. These selected ingredients exhibit many positive findings such as ability in reducing inflammation, sneezing, sore throat, carcinogenic activity specially in lungs. It also proves that most of these ingredients such as Retrofractamide – A act specially on lungs[13].

Discussion

Peenasa is a *Vata – Kapha* predominant disease[2] and with *Pitta dosha* vitiation can be correlated with Allergic rhinitis. It is mentioned in traditional system of medicine as *Diya peenasa, Sotu peenasa* or *Sem peenasa*[9]. Allergic rhinitis is an inflammation of the inside of the nose caused by an allergen, such as pollen, dust or flakes of skin from certain animals[7]. Though the main *dosha* involved in *Peenasa* are *Kapha* and *Vata*, involvement of *Pitta dosha* can also be seen. Bhava Prakasha mentions the symptoms of *Peenasa* clearly which can be used in proper diagnosis such as coated like feeling in nose, smoky sensation, dry and wet time to time, no sense of smell and sneezing [11]. Also, it is known to be a long-lasting disease with hereditary factors. Complications such as sinusitis, headache, hearing loss can be occurred due to untreated allergic rhinitis[3]. Other than the internal treatments for *Peenasa*, external treatments like application of oils like RT can be used to treat by considering the *Dosha* condition of the disease[2]. In Ayurveda and Traditional medical system, *Peenasa* is treated with both internal and external treatment modalities. Internal drug administration is aimed in systemic effect of medicines while external administration is mainly focused on the local effect of the drug. In the present novel treatment protocol for *Peenasa, Triphala kwatha* and *Triphala churna* was used as the internal treatment line and *Rathulunu thailaya* (RT) was used as the external treatment.

Application of RT on head (*Sheersha abhyanga*) helps in reducing headaches, stimulating the nervous system, reducing hair fall, hair greying and helps in removing accumulated phlegm in sinuses and in relaxation of mind. Administration of medicinal drugs through nasal cavity is known as *Nasya, Shiro virechana, Murdha virechana, shiro vireka, Nasya* or *Navana*[10]. *Virechana* means elimination of *Dosha* from body and *Shiro virechana/Murdha virechana* means elimination of morbid *Dosha* particularly from areas above the clavicle including the head region. *Nasya* can be classified under three main categories as *Rechana* (purification), *Tarpana* (nourishing) and *Shamana* (pacification). Charaka Samhitha mentions five types of *Nasya* as *Navana* (inhalation of drugs in form of nasal drops), *Avapeeda* (insufflation of drugs in thin paste form through nasal passage), *Dhmapana* (insufflation of drugs in powder from through nasal passage), *Dhuma* (inhalation of drugs in form of form of smoke) and *Pratimarsha* (application of medicated

Table 1: Internal treatment protocol

Drug	Dose	Route of Administration	Duration
Triphala kwatha	15ml (1 table spoon) b/d before meal morning and evening	Oral	04 weeks
Triphala churna	2.5g (½ tea spoon) b/d after meal morning and evening	Oral	06 weeks

Table 2: External treatment protocol

Drug	Method of administration	Duration	Time
Rathulunu thailaya (RT)	*Shiro abhyanga* (apply on head)	08 weeks	morning and evening
	Prathimarsha nasya (errhine therapy)	2nd and 4th week (14 days)	morning

Table 3: Clinical assessment before, during and after the treatment protocol

Signs and Symptoms	Before treatment	During treatment							
		1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	7th Week	8th Week
Frequent sneezing	+++	+++	+++	++	+++	++	+	+	----
Watery nasal discharges	+++	+++	++	++	+	+	+	---	---
Nasal congestion	+++	+++	+	++	+	++	+	+	---
Sore throat	+++	+++	++	++	+	++	+	+	---
Headache	++	++	++	+	---	+	---	---	---
Redness of Nasal mucosa	++	++	+	+	++	+	---	---	---
Itching of eyes	---	---	+	---	+	+	---	---	---
Other	Indigestion, flatulence. Pain and itching of nose	Indigestion, flatulence. Pain and itching of nose	Flatulence, Itching of nose	Flatulence, Itching of nose	---	---	---	---	---
Other effects after treatment	---	---		---		Sound sleep		Thickening of hair	

[More - +++, Moderate - ++, Less - +, Absent – ---]

Table 4: Ingredients used to prepare *Triphala kwatha* and *Churna* [12]

Common name, Sinhala and Sanskrit name	Botanical name	Family name	Parts used
Myrobalan (*Aralu/ Harithaki*)	*Terminalia chebula (Gaertn.) Retz.*	COMBRETACEAE	Fruit
Beleric (*Bulu/ Vibhithaka*)	*Terminalia bellirica (Gaertn.) Roxb.*	COMBRETACEAE	Fruit
Indian gooseberry (*Nelli/ Amalaki*)	*Phyllanthus emblica Linn*	PHYLLANTHACEAE	Fruit

Table 5: Ingredients used to prepare *Rathulunu thailaya* (RT)[12]

Common name and Sinhala name	Botanical name	Family name	Part/s used
Red onion (*Rathulunu/ Palandu*)	*Allium cepa* L.	AMARYLLIDACEAE	Fruit
Sesame oil (*Thala thel/ Thila*)	*Sesamum indicum L.*	PEDALIACEAE	Seed oil
Dried ginger (*Wiyali inguru/ Shunti*)	*Zingiber officinale* Roscoe.	ZINGIBERACEA	Rhizome
Pepper (*Gammiris/ Krushna*)	*Piper nigum* L.	PIPERACEAE	Seeds
Long pepper (*Thippili/ Pippali*)	*Piper longum* L.	"	"
Nutmeg (*Sadikkā/ Jathiphala*)	*Myristica fragrans* Houtt.	MYRISTICACEAE	"
Nutmeg cover (*Wasawasi/ Jāthiphala*)	"	"	Seed cover
Asafoetida (*Perumkāyam/ Hingu*)	*Ferula foetida* Linn.	UMBELLIFERS	Resin
Black cumin (*Kalu duru/ Kalajaji*)	*Nigella sativa L.*	RANUNCULACEAE	Seeds
White cumin (*Suduru/ Jeeraka*)	*Cuminum cyminum* L.	APIACEAE	"
Clove (*Karabu nati/ Lavanga*)	*Syzygium aromaticum* L.	MYRTACEAE	Flower buds

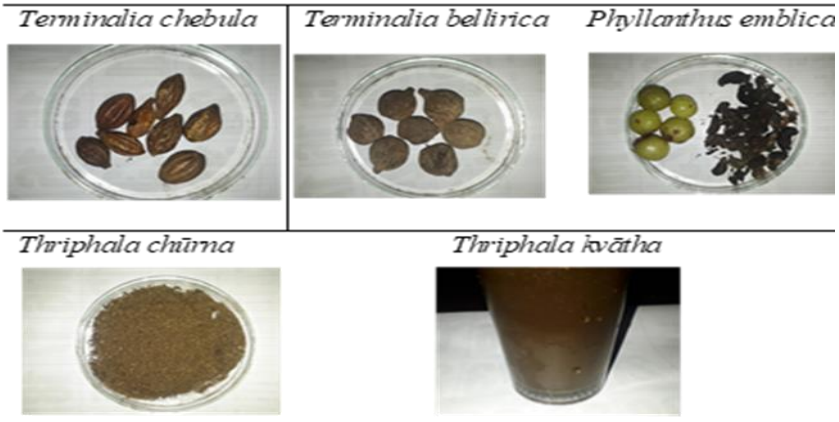

Figure 1: Ingredients of *Triphala kwatha* and *Churna*

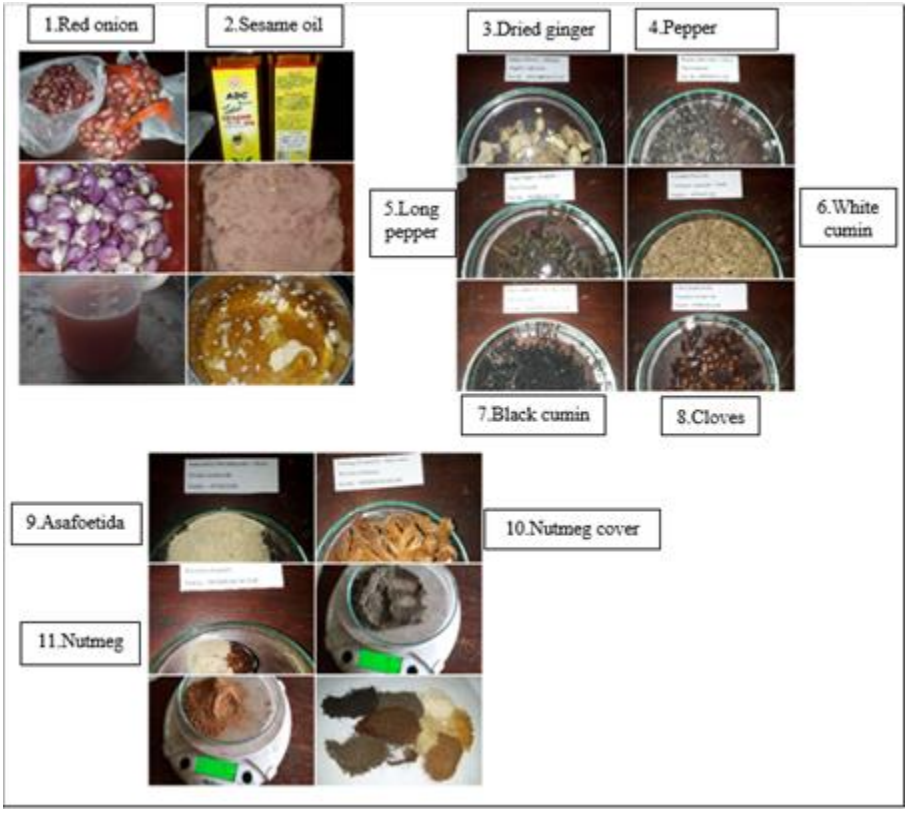

Figure 2: Ingredients of *Rathulunu thailaya* (RT)

Table 6: Pharmacodynamic properties of *Triphala* according to Ayurveda [13]

Ingredient	Ayurveda pharmacological properties (*Rasadi panchakaya*)				
	Rasa (taste)	*Guna* (quality)	*Virya* (potency)	*Vipaka* (final taste after digestion)	*Prabhava* (specific potency)
Terminalia chebula (Gaertn.) Retz. (*Aralu*)	*Kashaya* (astringent), *Amla* (sour), *Katu* (pungent), *Thiktha* (bitter), *Madhura* (sweet),	*Laghu* (light), *Ruksha* (rough)	*Ushna* (hot potency)	*Madhura* (sweet final transformation of digestion)	---
Terminalia bellirica (Gaertn.) Roxb. (*Bulu*)	*Kashaya* (astringent)	*Laghu* (light), *Rūksha* (rough)	*Ushna* (hot potency)	*Madhura* (sweet final transformation of digestion)	---
Phyllanthus emblica Linn (*Nelli*)	All 5 tastes except *Lavana* (salty taste)	*Laghu* (light), *Ruksha* (rough)	*Sheetha* (cold potency)	*Madhura* (sweet final transformation of digestion)	---

Table 7: Pharmacodynamic properties of *Rathulunu thailaya* (RT) [14]

Rasadi panchakaya **(Pharmacodynamic properties)**	**Percentage**
Rasa (taste)	*Katu* (pungent) - 90.90%, *Tiktha* (bitter) - 45.45%
Guna (quality)	*Laghu* (light) – 81.81%, *Theekshna* (sharpness) - 63.63%
Virya (potency)	*Ushna* (hot) - 100%
Vipāka (final taste after digestion)	*Katu* (pungent) - 72.72%
Prabhāva (specific action)	*Keshya* (good for hair) – 1/11 = 9.09% [*Thila*/ sesame]

oil in nostrils[22]. RT was prescribed for the patient as *Prathimarsha nasya* which can be considered as a *Shamana nasya* in curing the disease. *Nasya* has also been mentioned as a *Dina charya* (daily routine) to maintain healthy life in Ayurveda[23].

Triphala is a drug formulation which can be used in different preparation methods like *Kwatha, Churna* etc. as a single drug regimen and said to be having the ability of pacifying the vitiated *Tridosha,* in *Peenasa.* RT had been used for many years since antiquity in treating *Peenasa* by Ayurveda and traditional medical practitioners of Sri Lanka. Due to the effectiveness in treating *Peenasa,* the oil is known as *Peenas thailaya*. In the present study, two (02) preparations of *Triphala* formulation as *Triphala kwatha* (preserved form of decoction) and *Triphala churna* (medicinal powder) was administered internally. Analysis of *Rasadi panchakaya*, of both these drug formulae revealed that most of the ingredients were having *Katu* (pungent), *Thiktha* (bitter) tastes, *Laghu* (light), *Theekshna* (sharp) properties, *Ushna veerya* (hot potency) and *Katu vipaka* (pungent final taste after digestion)[13] which help to pacify vitiated *Kapha dosha*. Although literature mentions RT can be used as an internal medicine [12], in this study RT was applied on head (*Sheersha abhyanga*) and Errhine therapy (*Prathimarsha nasya*) which showed the effectiveness.

Literature search on chemical constituents of *Triphala*[24] and *Rathulunu thailaya*[14] revealed many medicinal properties like anti-inflammatory, anti-infective, anti-microbial and anti-cancerous effects and presence of anti-oxidants which are useful in treating diseases like *Peenasa*. The study showed the effectiveness of this novel treatment protocol in managing *Peenasa* (Allergic rhinitis) within a short period of time like two (02) months. Further, the patient disclosed about the healthiness and thickness of hair after the application of RT. *Keshya prabhava*[13] of sesame is the probable reason for the healthiness and increased thickness of hair at the end of the treatment.

Though there are many commonly practiced drugs in managing *Peenasa,* the novel treatment plan which consisted *Triphala kwatha, Triphala churna* and of *Rathulunu thailaya* (RT) was utilized in the management of *Peenasa* in the present study. Although usage of *Triphala* drug formulation is not commonly practiced in the management of *Peenasa,* along with the external application of *Rathulunu thailaya* (RT), novel treatment protocol gave successful results in the management of *Peenasa* without reporting any adverse effects.

Conclusion

Peenasa can be correlated with Allergic rhinitis according to Allopathic medicine and *Diya peenasa*, *Sotu peenasa* or *Sem peenasa* according to Traditional system of medicine in Sri Lanka. Reduction and pacification of phlegmatic condition along with other complaints like flatulence and indigestion was recorded as added findings in this study. Even though the novel treatment protocol was different to the normally practiced treatment plans in Ayurveda, successful results were obtained for the management of *Peenasa* within a short duration without any reported side effects. Hence, the novel treatment protocol proved the safety and effectiveness in reducing clinical features of *Peenasa* (Allergic rhinitis).

Suggestions

Chemical analysis can be done to isolate active ingredients and secondary metabolites to analyze pharmacological properties and actions of the drugs used in the study. Advanced clinical trials as Randomized control trials (RCT) can be done to scientifically test the therapeutic efficacy of this novel treatment protocol in the management of *Peenasa.*

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Evaluation of *In Vitro* Anti-inflammatory and Antibacterial Activities of Leaves, Roots and Combination of Leaves and Roots Extracts of Plant *Magnolia figo*

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Abstract

Presently there is a rising demand for novel and potent anti-inflammatory drugs of plant origin owing to the lesser amount of side effects compared to Non-Steroidal anti-inflammatory Drugs. On the other hand, microbial resistance to conventional antimicrobial agents has increased during the last three decades. This study aims to overcome these challenges by investigating an herbal plant application. It was designed to evaluate in vitro anti-inflammatory and antibacterial activity of methanolic extracts of leaves, roots and combination of both leaves and roots extracts of *Magnolia figo*; a native Chinese medicinal plant. In vitro anti-inflammatory property was determined using heat-induced protein (egg albumin) denaturation test using diclofenac sodium as the positive control. Antibacterial activity of the methanolic extract was evaluated against *Escherichia coli* (ATCC® 25922TM) and *Staphylococcus aureus* (ATCC® 25923TM) using the cylinder plate method. As a result, considerable dose-dependent anti-inflammatory activities were detected in the combination extract (IC50 =1.819 µg/mL) when compared to the positive control (diclofenac sodium) (IC50 =4.337 µg/mL). Among the extracts, the highest effect against *E. coli* was exhibited by the combination extract (diameter of inhibitory zone =15.30 mm). None of the extracts showed a positive antibacterial effect against *S. aureus*. Phytochemical investigations of extracts indicated the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, glycosides and steroids. This was concluded a significant result elicited by the combination of plant parts confirm that *M. figo* is a medicinal plant that can be used to develop novel anti-inflammatory and antibacterial agents.

Keywords: Anti-inflammatory, Antibacterial, Phytochemical analysis, *Magnolia figo*, *Escherichia coli*, *Staphylococcus aureus*

Introduction

Traditional herbal remedies are more acceptable in most ethnic societies as compared to allopathic medicines because they are considered to be the safest approach to treat diseases with the least side effects on human health. Nature has provided abundant plant wealth for all living creatures which create a medicinal virtue. The total number of the 252 drugs in the WHO essential medicine list,11% of medicines are exclusive of plant origin[1].The bioactive compounds of medicinal plants are used as antidiabetic, chemotherapeutic, anti-inflammatory, anti-arthritic agents where there is no satisfactory cure in modern medicines[2].

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Hence, the present study aims to determine the anti-inflammatory and antibacterial activity of selected plant parts of *Magnolia figo*.

Inflammation is the bodily response to an injurious stimulus evoked by a wide variety of noxious agents, for example, physical trauma, noxious chemicals or microbial agents[3]. Studies have shown that single or multiple bacterial origins can be the cause for inflammation where the microbial invasion stimulates acute inflammation in to a chronic inflammation[4].The commonly used drugs for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) which have several side effects especially gastric irritations leading to the formation of gastric ulcers, cardiovascular risks and hormonal imbalance. Instead of side effects causing NSAIDs, the rich wealth of the plant kingdom has been used to represent a novel source of compounds with anti-inflammatory activities[3]. The denaturation of protein is one of the causes of inflammation. In certain rheumatic diseases, the production of auto antigens maybe due to the denaturation of proteins. Anti-inflammatory drugs are known to inhibit the denaturation of proteins. Therefore, agents that can prevent denaturation could be used for the development of anti-inflammatory drugs and anti-arthritic drugs[5].

The emergence of new infectious diseases, the resurgence of several infections and the increase in bacterial resistance have created the necessity for studies directed towards the development of new antimicrobial agents. Most bacteria, fungi and viruses have a lesser ability to develop resistance against the botanicals. Alteration of target sites, active efflux of drugs and enzymatic degradations are the strategies employed by the pathogenic bacteria to develop intrinsic resistance to antibiotics. Secondary metabolites of plants such as alkaloids, tannins, polyphenols etc. could act as potentials for antimicrobials and resistance modifiers. Plant extracts can bind with protein domains leading to modification or inhibition of protein-protein interactions[6].

The Magnoliaceae is a family of about 220 species of deciduous or ever green trees and shrubs native to Asia and America with large showy flowers. Based on the pharmacological actions Magnoliaceae plants report three categories of features: biochemical (37.5%), cardiovascular (20.8%) and CNS pharmacology (25.0%)[7].

Magnolia figo plant which belongs to the Magnolia (Family Magnoliaceae) genus are arboreous plants growing in the temperate zone. Though it grows in China, now it is cultivated both in the hill-country and mid-country in Sri Lanka. It is commonly called as banana shrub, because of the heavy, sweet banana scent of its purple flower. The seeds and flowers are used for making preparations for strengthening sexual virility. The bark is a stimulant, expectorant, astringent and the root is a purgative. There is no scientific evidence available on *M. figo* leaves and roots corresponding to their in vitro evaluation of anti-inflammatory activity and antibacterial activity. The leaves of this shrub contain alkaloids, magnolamine, magnoline and tetrandrine[8].

This study is designed to evaluate the anti-inflammatory and antibacterial action of *M. figo* plant parts including leaves and roots as the first approach of developing newer drugs that can be used as anti-inflammatory drugs with fewer side effects and antibacterial drugs with less microbial resistance. Furthermore, phytochemical screening of *M. figo* plant parts and the comparison of their anti-inflammatory and antibacterial activities will be a remarkable approach for the development of a novel therapeutic drug.

Materials and Methods

Study Location

The study was carried out in the Chemistry Laboratory and Research Laboratory, General Sir John Kotelawala Defense University, Rathmalana and Pharmacy Skills Laboratory, Faculty of Allied Health Sciences in General Sir John Kotelawala Defense University, Werahera, Sri Lanka.

Plant collection and authentication

About 800g of each matured, fully expanded leaves and roots of *M. figo* were collected from Peradeniya, Kandy in fresh condition at day time after studying

the morphological and organoleptic properties carefully. The plant voucher specimens (NH/ BOT/4/2019-51(II)) were deposited according to the herbarium rules and regulations and authenticated by National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. The collected plant materials were thoroughly cleaned using running tap water and air-dried until a constant weight was obtained.

Preparation of crude plant material extracts

Powdered samples were used for the extraction procedure. The methanolic extract was obtained by cold maceration. The alcohol was distilled off from the filtrate under reduced pressure to obtain a dry residue. This extract was stored in a desiccator at a low temperature until the actual experiment was done[5].

Heat-induced protein denaturation test

A concentration series of plant extracts were compared with the same concentration of the positive control for the anti-inflammatory activity. Diclofenac sodium was used as the standard anti-inflammatory drug. A similar volume of double distilled water was used as the negative control. Egg albumin was obtained by centrifugation of diluted fresh chicken egg white and samples were added for the preparation of the reaction mixture. The pH of the egg albumin was adjusted by adding freshly prepared phosphate buffer saline (PBS, pH=6.4). Albumin (270µl) and 30µl of different concentrations of plant samples were inserted into each well from rows of A to C and columns of 96 well flat bottom microtiter plate and triplicated the reaction mixture. Albumin (270µl) and 30µl of negative control samples were added into each well in column D. Albumin (270µl) and 30µl of positive control samples were inserted into each well in rows of E and F and columns. Albumin (300µl) was inserted into each well in rows of G and H columns. Mixtures were incubated at 37°C (37±2°C) for 10 to 15 minutes. The denaturation process was induced by increasing the temperature gradually up to 57° Cand kept for 5 minutes. Samples were allowed to cool down to room temperature at 30°C. After cooling down, the absorbance was measured at 660nm using an ELISA plate reader. The percentage inhibition of protein denaturation for each sample was calculated by using the absorption readings according to the equation.

Percentage of inhibition = 100*[Vt/Vc-1]

Where Vt = absorbance of the test sample and Vc = absorbance of control[5]

The dose-response curve of each sample was compared with each other and the positive control. The 50% inhibition (IC50) of extract drug concentration was determined by plotting percentage inhibition with respect to control against treatment concentration.

Statistical analysis

The results are presented as mean,±SEM (Standard Error of Mean) and ±SD (Standard Deviation) was calculated by descriptive statistical analysis and correlation analysis using Statistical Package for Social Sciences (SPSS) version 25. A significant level was set up at $p<0.05$. The concentration dependencies and IC50 value (half maximal inhibitory concentration) were calculated by affecting nonlinear regression using the Graph pad Prism 9 statistical software package.

Cylinder plate antibiotic assay method

Stock cultures of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were obtained as common wound infecting bacteria. A serial dilution series was prepared by re-dissolving crude extract in Dimethyl sulfoxide (DMSO) starting from 1500µg/mL filtrate up to 250µg/mL. In this study, gentamycin (50 µg/ mL) was used as the positive control[9]. It was prepared by using a commercially available gentamycin (40 mg/mL) IV injection vial. Sub cultures were prepared using bacteria (*Escherichia coli* and *Staphylococcus aureus*) in nutrient agar slants and incubated at 37°C for 24 hours to obtain pure bacterial colonies to prepare the bacterial broths. Two to three bacterial colonies were obtained from subcultures using a sterile inoculating loop and dissolved in 5mL of 0.9% sterile normal

saline under aseptic conditions. Then the bacterial suspension was compared with previously prepared McFarland turbidity standards. Turbidity of bacterial suspension was adjusted to get equivalent with 0.5 McFarland standard. Turbidity of 0.5 McFarland standard was equivalent to that of a bacterial suspension of 1.5 × 108 CFU/mL.

Two bacteria suspensions (1 mL) were added to the 45mL nutrient agar containing flasks. Bacteria suspension was added into agar at 40°C of temperature by using a sterile 1mL pipette under sterile conditions. Cylinders were placed according to a template positioned under the Petri plate which made uniform gaps between cylinders. All the procedures were carried under strict aseptic conditions[10]. Gentamycin (50 µg/ mL) was used as the positive control and DMSO was used as the negative control. Each step of the experiment was repeated as triplicates. Plates were kept at room temperature (25°C – 30°C) for 2 hours to allow diffusion of extracts into the agar. All the plates were incubated at 37°C in an incubator for 24 hours[11]. After incubation, the diameter of the inhibition zones was measured using a vernier caliper.

Phytochemical screening of the M. figo roots and combination extracts

Phytochemical screening of roots and a combination of roots and leaves were done using previous study methods. Accordingly, Flavonoids (Alkaline Reagent test)[12], Carbohydrates (Molisch Reagent test)[13], Tannins (Braymer's test)[14], Saponins (Froth test)[12], Alkaloids (Wagner's test)[15], Glycosides (Keller-Kiliani Test)[14], Phenols (Ellagic acid Test)[14], Terpenoids (Salkowski test)[13], Steroids (Liberman Burchard test)[13], amino acids and proteins (Ninhydrin Test)[15] levels in the selected plant parts of *M. figo* were detected.

Results

Anti-inflammatory effect of methanolic leaves, roots and combination (leaves and roots) extracts of Magnolia figo

The combination extract of leaves and roots showed the highest percentage of inhibition when compared to the other two extracts. The leaf extract showed the next highest percentage inhibition at the 1000 µg/mL concentration. Concentration at 31.25 µg/mL also, the second-highest percentage inhibition was shown by the leaf extract. At the concentration of 0.5 µg/mL, the extract of the root has shown the second-highest percentage inhibition among the three types of extracts (Table 1).

Figure (1) shows that, with the increasing log concentrations of *M. figo* leaves extract, percentage inhibitions were also increasing.

Combination extract and leaves extract showed higher potencies compared to diclofenac sodium. Root extract showed lower potency compared to diclofenac sodium (Table 2).

Antibacterial effect of methanolic leaves, roots and combination (leaves and roots) extracts of Magnolia figo against Escherichia coli

According to the results (Table 3), the highest antibacterial effect against *E. Coli* was expressed by methanolic roots and leaves combination extract, with a concentration of 1500µg/mL and the inhibitory zone was 15.30mm. The lowest antibacterial effect against *E. Coli* was expressed by roots extract while leaves extract showed a moderate effect. Mean inhibition zones of extracts with a concentration of 1500µg/mL of leaves and roots extracts were 15.10mm and 14.45mm respectively.

All the extracts of *M. Figo* showed the highest antibacterial effect when the concentration of the sample was 1500µg/mL. All the extracts exhibited an antibacterial effect against *E. coli* in all the five concentrations (1500µg/mL, 1000µg/mL, 750µg/mL, 500µg/mL, 250µg/mL) tested. None of the *M. figo* plant extracts exhibited a positive antibacterial effect against *S. Aureus* as none of the extracts showed an inhibitory zone compared to the negative control. All values were around 10mm (Table 4).

According to the data obtained by EC50 values and dose-response curve, methanolic combination extract of *M. Figo* exhibited the highest efficacy and highest potency and at the same time the methanolic roots extract and leaves extract exhibited lower potencies than combination extract (Figure 2).

According to the above studies (Table 5), it was found that $p<0.05$ which indicates that the data is significant. Further, all R^2 values were found to be nearly 1 and it shows that there is a positive correlation between the zone of inhibition and concentrations of all three plant extracts.

Phytochemical study results indicated the presence of alkaloids, flavonoids, carbohydrates, phenols, tannins, saponins, protein and amino acid, terpenoids, glycosides and steroids in the extracts of the combination of leaves and roots of *M. Fig o*plant whereas roots confirm the absence of saponins, glycosides and terpenoids (Table 6).

Discussion

Studies conducted for the evaluation of sunscreen activity and antioxidant activity of methanolic leaf extract of *M. figo*; phytochemical screening indicate that leaf extracts are rich in alkaloids, phenols, tannins, terpenoids, flavonoids, steroidal glycosides and saponins.The results concluded that leaf extract of *M. Figo* possesses marked antioxidant activity and sunscreen activity which exhibit its potential use for the prevention of oxidative stress[16].

According to the literature reviews, the antibacterial activity and anti-inflammatory activity of methanolic leaf and roots extracts of *M. Figo* were not evaluated in Sri Lanka in the past. The study was conducted using the methanolic extracts of the plant parts of *M. Figo* to overcome the problems that occurred in conventional medicines used in inflammations and bacterial infections. The anti-inflammatory study was carried out by using the egg albumin denaturation test. As the egg albumin was not denatured, it was confirmed that those plant extracts contain phytoconstituents which have anti-inflammatory properties. The absorbance of denatured samples was analyzed by using an ELISA plate reader. The absorbance of the plant sample was compared with the diclofenac Na drug sample and inhibition of albumin denaturation was identified. Variations in hydrogen, hydrophobic, electrostatic and disulphide bonding would have taken place due to the mechanisms of denaturation[17]. PBS was used to maintain pathological pH (pH 6.2-6.5).

In this investigation, the methanolic combination extract of *M. figo* exhibited greater inhibition of protein denaturation than the anti-denaturation effect of the standard drug diclofenac sodium. It is noticeable that except for roots extract, all other plant extracts showed a higher potency compared to diclofenac sodium. Diclofenac sodium exhibits an IC50 value of 4.337 µg/mL and a high R2 (0.9220) indicating a strong positive relationship with the inhibitory percentage and log concentrations. On the other hand, the combination extract curve showed a similar pattern to diclofenac sodium. On the whole, results show that combination extract overcomes the action of the reference drug. It showed a higher or synergistic anti-denaturation effect compared to the other two extracts of leaves and roots. The methanolic leaves, roots and combination extracts showed marked dose - dependent anti-denaturation effects indicating potent anti-inflammatory activity with an IC_{50} value of 4.132µg/mL, 6.519µg/mL, and 1.819µg/mL respectively (IC_{50} value of diclofenac sodium is 4.337µg/mL). There was a strong positive statistically significant ($R^2=0.8307$, $p<0.001$) correlation between concentration and percentage inhibition by leaves extract of *M. figo*. There was a moderate positive statistically significant ($R^2= 0.4422$, $p<0.001$) correlation between concentration and percentage inhibition by roots extract of plant *M. figo*. There was a relatively strong positive statistically significant ($R^2= 0.5684$, $p<0.001$) correlation between concentration and percentage inhibition by combination extract of plant *M. Figo* ($R^2= 0.9220$, $p<0.001$ of diclofenac sodium).

Table 1: Percentage inhibition for extract samples of *M. figo* plant parts and reference drug

Concentrations (µg/mL)	Leaves	Roots	Combination	Reference Drug (Diclofenac Na)
1000	86.365±12.2	61.389±7.5	97.895±25.3	96.128±0.3
500	85.299 ± 7.3	58.977±9.9	93.468±8.6	95.674±3.6
250	61.293 ± 1.3	56.345±6.5	82.418±19.8	89.002±1.0
125	61.151 ± 3.5	66.910±3.1	81.748±15.8	90.377±11.1
62.5	62.225 ± 2.3	41.983±5.7	79.179±12.0	76.992±0.4
31.25	64.140 ± 0.76	47.908±16.6	66.236±10.1	70.808±3.5
15.625	36.679 ± 6.62	43.465±1.1	68.74±16.2	68.732±1.8
7.8125	20.282 ± 0.76	20.887±2.1	54.684±11.7	64.329±0.7
3.9	9.877± 1.32	16.387±0.1	38.86±12.367	41.518±11.2
1.95	6.363 ±5.51	27.125±2.8	25.239±23.3	20.945±0.1
1	0.324 ±0.76	31.956±1.2	21.421±17.2	15.129±6.1

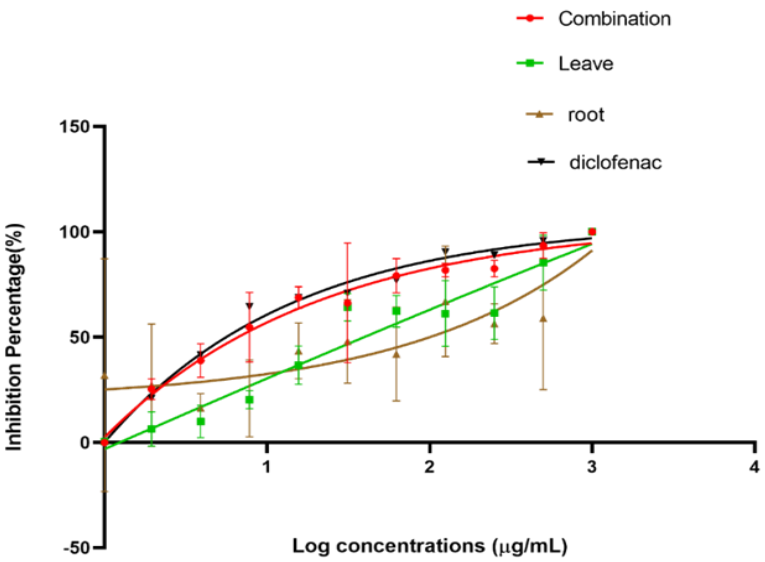

Figure 1: Dose-response curves for anti-inflammatory properties of the *M. figo* plant leaves, roots and combination extracts and reference drug (diclofenac sodium) based on inhibition percentage.

Table 2: Details of the dose-response curves of reference drug and *M. Figo* leaves, roots and combination (leaves, roots)

Tabular results	**Leaves**	**Roots**	**Combination**	**Reference drug (Diclofenac Na)**
IC_{50}(µg/mL)	4.132	6.519	1.819	4.337
R^2	0.8307	0.4422	0.5684	0.9220
P value	<0.0001***	<0.0001***	<0.0001***	<0.0001***

*** P<0.001

Table 3: Antibacterial effect of methanolic leaves, roots and combination extracts of *M. Figo* against *E. Coli.*

Concentrations (µg/mL)	Zone of inhibitions of *Magnolia figo* plant parts		
	Leaves	Roots	Combination
1500	15.10±0.12	14.45±0.35	15.30±0.04
1000	14.14±0.34	14.11±0.13	15.31±0.18
750	13.21±0.20	12.52±0.15	14.51±0.50
500	12.20±0.25	12.63±0.15	13.47±0.17
250	10.86±0.22	11.47±0.21	12.92±0.60
Positive control	30.84±1.19	28.61±2.06	29.78±1.98
Negative control	10.18±0.05	10.21±0.34	10.33±0.30

(Data is expressed as, mean inhibitory diameter ±SEM/ Positive Control-Gentamycin 50 µg/ml/ Negative control - DMSO (Dimethyl Sulfoxide))

Table 4: Antibacterial effect of methanolic leaves, roots and combination extracts of *M. figo* against *S. aureus.*

Concentrations (µg/mL)	Zone of inhibitions of *Magnolia figo* plant parts		
	Leaves	Roots	Combination
1500	10.33 ± 0.3	10.66 ± 0.2	9.96 ± 0.2
1000	10.15 ± 0.3	10.61 ± 0.2	10.22 ± 0.3
750	10.28 ±0.5	10.52 ± 0.2	10.44 ± 0.4
500	10.54 ± 0.1	10.36 ± 0.3	10.34 ± 0.3
250	10.12 ± 0.1	10.51 ± 0.1	10.26 ± 0.2
Positive control	30.84 ± 1.2	30.16 ± 2.0	28.37 ± 2.1
Negative control	10.18 ± 0.1	10.22 ± 0.8	10.33 ± 0.3

(Data is expressed as, mean inhibitory diameter ±SEM/Positive control- Gentamycin 50 µg/ml/Negative control - DMSO (Dimethyl Sulfoxide))

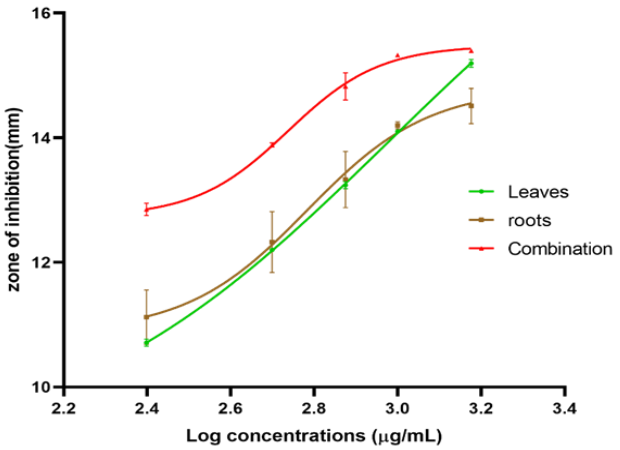

Figure 2: Dose-response curves of methanolic leaves, roots and combination extracts of *M. figo* against *E. Coli*

Table 5: Details of the dose-response curves of plant extracts against *E. Coli.*

Escherichia coli	**Leaves**	**Root**	**Combination**
EC_{50} (µg/mL)	946.5	606.7	536.2
Log EC_{50}	2.976	2.783	2.729
p value	0.0001*	0.0002*	0.0005*
R^2	0.9994	0.9521	0.9986

*p<0.05

Table 6: **Phytochemical analysis of *M. figo* plant.**

Phytochemical	**Test**	**Results**	
		Root	**Combination (leaves+roots)**
Flavonoids	Alkaline Reagent test	+	++
Carbohydrates	Molisch Reagent test	+++	+++
Tannins	Braymer's test	+	+++
Saponins	Froth test	-	++
Alkaloids	Wagner's test	+	+++
Glycosides	Keller-Kiliani test	-	++
Phenols	Ellagic acid test	+	+++
Aminoacids and proteins	Ninhydrin test	+++	+++
Terpenoids	Salkowski test	-	+
Steroids	Liberman Burchard test	++	+

(Mild presence: (+) / Moderate presence: (++) / High presence: (+++)

Thus, methanolic extract of leaves shows a much higher correlation between concentration and percentage inhibition than roots and combination extracts of the plant. From the above results, it is concluded that the methanolic combination extract of *M. Figo* possesses considerable anti-inflammatory activity *invitro* and this is a novel finding. Anti-inflammatory activity is likely to be mediated via the synergistic effect of flavonoids, alkaloids, tannins, saponins, phenols, steroids, glycosides and terpenoids[18].

According to the findings, the presence of higher amounts of phenols, flavonoids, tannins, saponins, terpenoids, steroidal glycosides and alkaloids in combination extract was shown compared to the other two extracts of the plants. As such, it can be assumed that the above secondary metabolites caused the synergistic effect in the combination extract. A wide range of flavonoids with various chemical structures was associated with different anti-inflammatory effects by significantly inhibiting several numbers of inflammatorymediators[19]. Terpenoids also possess significant analgesic and anti-inflammatory

activities. Such activity has been attributed to the ability to inhibit phospholipase A_2 and there by ultimately blocking the metabolism of arachidonic acid. Several alkaloids may also prevent inflammation by blocking the metabolic pathway of arachnoid acid[20].

Polyphenols reduce blood pressure, inflammation and work as oxidative markers and also, they prevent endothelial dysfunction, they are antithrombotic, and they act as vasodilators. They also inhibit the proinflammatory activity of Cyclooxygenase (COX), Lipoxygenase (LOX) and Inducible Nitric Oxide Synthase (iNOS)[21]. Tannins could affect the inflammatory response via free radical scavenging properties and inhibition of iNOS in macrophages, whereas saponins inhibit pain and inflammation via Nitric Oxide (NO) inhibition[18]. Steroidal glycoside called tomatine proves to be effective as an anti-inflammatory agent in humans without exerting the serious side effects observed with both the steroid and non-steroid anti-inflammatory drugs, it may be used in the treatment of chronic inflammatory diseases[22].

Gram-negative *E. coli* (ATCC 25922) andgram-positive *S. aureus* (ATCC 25923) were used to evaluate the antibacterial activity of leaves, roots and combination (leaves and roots) extracts of plant *M. Figo* using cylinder plate method. Gentamycin; the positive control of our study is a broad-spectrum bactericidal agent against many aerobic gram-negative bacteria, but has lower activity against most gram-positive organisms with the exception of staphylococci. The concentration of 50µg/mL was prepared because the gentamycin concentration range for antibiotic sensitivity testing should be within 0.003-64µg/mL. The results of the study show that the methanolic combination extract exhibited the highest zone of inhibition (15.30mm) against gram-negative *E. coli.* Roots extract of *M. Figo* exhibited the lowest inhibition (14.45mm) against gram-negative *E. coli.* Leaves extract exhibited moderate inhibition (15.10mm) against *E. coli*. All three methanolic extracts have no positive inhibition against gram-positive *S. aureus.* (diameter of the well in the agar plate=8mm).

The results revealed that 1500µg/mL concentration of all three extracts had the highest zones of inhibition. Accordingly, concentrations of the extracts have shown a positive correlation with the zone of inhibition against *E. coli* with R^2 values approximately equal to 1. Extracts at the concentration of 1500µg/mL have shown their zone of inhibition approximately between 15.30mm-14.45mm which is much closer numerically. Further, they have shown a statistical significance ($p<0.05$) between their mean zones of inhibition against *E. coli* at a concentration of 1500µg/mL. Yet, they have shown a mean zone of inhibition which is less than the positive control (approximately 30mm). Besides, they have not exhibited an effective antibacterial activity at the concentration of 250µg/mL. None of the concentrations have shown a positive antibacterial response against *S. aureus*. Yet, the positive control has shown a zone of inhibition approximately equal to 30mm. Hence, methanolic extracts of leaves, roots and a combination of *M. Figo* are not effective as an antibacterial agent against *S. Aureus.* Analyzing the data obtained from the dose-response study, the highest EC_{50} value (946.5µg/mL) against *E. coli* is exhibited by methanolic leaves extract whereas the lowest EC_{50} value (536.2µg/mL) was obtained from methanolic combination extract. Methanolic combination extract has shown the highest antibacterial potency and methanolic leaves extract has the lowest antibacterial potency against *E. coli.*

According to these results, methanolic extracts of leaves, roots and the combination of *M. Figo* have shown a positive antibacterial response against gram-negative bacteria *E. coli* (ATCC 25922) and negative antibacterial response against gram-positive bacteria namely *S. aureus* (ATCC 25923). This indicates that leaves, roots and combination of *M. figo* extracts may possess a gram-negative antibacterial spectrum. Thus *M. figo* plant has the potential in the development of a novel antibacterial medicine with no antibiotic drug resistance.

The highest effect of anti-inflammatory activity was shown by the combination extract of *M. Figo* followed by its leaves extract and the root extract and

the highest effect of antibacterial activity was also shown by the combination extract of *M. Figo* followed by its root extract and leaves extract**.** The variable presence of phytoconstituents in different parts of the plant such as roots and leaves must have been the reason for the above observations**.**

M. figo roots extract showed a high presence of carbohydrates, amino acids and proteins with the moderate presence of phytochemicals such as steroids**.** Flavonoids, saponins and glycosides were present in the combination extract as moderately present phytoconstituents**.** On the whole, it can be concluded that the combined extract of *M. Figo* is a richer source of phytoconstituents than the leaves and roots extracts which would rise as a good antibacterial and anti-inflammatory activities**.** Flavonoids and phenols exert their antibacterial activity via interactions with proteins on the bacterial cell wall and thereby disrupting their function and integrity**.** Saponins also have been shown to induce antibacterial actions through a membranolytic action by increasing the permeability of bacterial cell wall[23]**.** Alkaloids are also believed to elicit antimicrobial and trypanocidal activity by inhibition of protein biosynthesis and by interaction with neuroreceptors**.** In the near future, it can be expected that phytochemicals-based drugs will be an object of growing interest for inflammatory and bacterial related diseases**.**

Conclusion

This study showed that methanolic extracts of *M. figo* plant parts (leaves, roots) have marked *in vitro* dose-dependent anti-inflammatory activity and antibacterial activity. The anti-inflammatory activity of methanolic leaves and combination extracts of the plant were more potent than the reference drug. *M. figo* extracts showed marked antibacterial activity against *E. coli*, but not as effective as the reference drug (gentamicin). These anti-inflammatory and antibacterial activities may be mediated through the synergistic effect of the secondary metabolites present in the *M. figo* plant. Further studies are necessary to establish the mechanism of action and ascertain the active constituents responsible for the pharmacological activities of the extracts of *M. figo* plant.

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Conflict of interest

Authors had declared that they have no conflict of interest.

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In-vitro Anti-urolithiatic Evaluation of Methanolic Extract of *Salacia reticulata*

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Abstract

Urolithiasis can be defined as the process of forming stones in the kidney, bladder and urinary tract. It is found that 1 in 20 people develop kidney stones at some stage in their life time. Treatment includes relief of pain, hydration and antibiotics. Although many advanced allopathic medicines are available in the modern world, most people have been using the herbal medical treatments owing to its less side effects and low cost. *Salacia reticulata* is one of the herbs used to cure the urinary stones in the human body. This research was conducted to evaluate *In-vitro* anti-urolithiatic activity of *Salacia reticulata* stem extract on experimentally prepared calcium oxalate stones. Methanolic plant extract was prepared by using the soxhlet apparatus. Artificial stones of calcium oxalate were prepared by homogenous precipitation method. Semi-permeable membrane bags were prepared from chicken eggs. One sample of negative control, four samples of positive control with 10, 20, 30, 40 mg of standard drug cystone and four other samples with 10, 20, 30, 40 mg of methanolic extract of *Salacia reticulata* were prepared separately. The prepared samples were incubated for 7 hours at 37 ^{o}C in pH 7.4. Titrimetric method was followed in order to determine the anti-urolithiatic activity. The mean (±SD) dissolution percentage of extract of *Salacia reticulata* on experimentally prepared calcium oxalate for 10, 20, 30 and 40mg were 34.8, 38.6, 40.4 and 45.0% respectively. The dissolution percentage significantly increased with concentration. Dissolution percentages of cystone on artificial calcium oxalate stones for 10, 20, 30, 40 mg were 64.8, 70.6, 75.8 and 79.6% respectively. Both the methanolic extract of *S. reticulata* and the standard drug has a positive correlation with its concentration. The results of this study have confirmed the long history of the use of *Salacia reticulata* in traditional medicine for the treatment of Urolithiasis.

Keywords: *Salacia reticulata*, Anti-urolithiatic, Cystone, Methanolic extract.

Introduction

Kidney stone can be described as a formation of crystal within the kidneys, affecting 12% of the world population. Urolithiasis is one of the most common diseases of the urinary tract that has been afflicting human kind since ancient times[1]. Recurrent stone formation is probably the most important problem in the after-care patients who have undergone operations for renal and ureteric calculi.

The most common type of kidney stone is calcium oxalate formed at Randall's plaque on the renal papillary surfaces. 50% of these stones are pure calcium oxalate, 5% is calcium phosphate and 45% is a mixture of both. Magnesium phosphate represents 15%-20%, cystine represents 1% and uric acid represents 10% of the stones. The kidney stones are mostly calcium oxalate, 86%[2].

The causative factors for the stone formation are of huge range including epidermiological, biochemical and genetic[3]. Urolith formation is multifactorial which may relate to diet, urinary tract infection, altered urinary solutes and colloids, decreased urinary drainage and urinary stasis, prolonged immobilization, Randall's plaque and microliths

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etc.[4]. The mechanism of stone formation is a complex process. It results from several physicochemical events including supersaturation, nucleation, growth, aggregation and retention of kidney stones constituents within tubular cells[5].

Urolithiasis has a reoccurrence rate of 70%-80% in males and 47%-60% in females, usually more frequent in men than women and rare in children[6]. But the current population shows high kidney stone disease tendency in children as well.

Although exact data are not available about the kidney stones disease in Sri Lanka, nearly 3000 patients are subject to Extra-Corporeal Shock Wave Lithotripsy (ESWL) for a year, however only in Sri Lankan National hospitals[7]. This information shows that the gravity of kidney stone disease in Sri Lanka. Factors like quality of drinking water, climate and different dietary habits are the risk factors for the formation of kidney stones in Sri Lanka.

Various treatment methods have evolved over the years for the kidney stone disease, discrepancies exist regarding the efficacy of the treatment options. Surgical operation, lithotripsy and local calculus disruption using high power laser are widely used to remove the calculi.

Many remedies have been employed to treat renal stones and most of them were from plants and proved to be useful. In Ayurveda and Folklore medicine, many herbs are used in the treatment of urolithiasis [8]. The discoveries of *in-vitro, in-vivo* and clinical trials show that phytotherapeutic agents could be useful as either alternative or adjunct therapy in management of urolithiasis.

Medicinal plants are major parts of traditional systems in developing countries. Herbal medicine is defined as the branch of science where plant used formulations are used to ease the diseases. It is known as Botanical medicine or Phytomedicine. Many diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries[9]. Medicinal plants which form the backbone of traditional medicine have been the subject of very intense pharmacological studies in the last few decades.

Ayurveda, Siddha, Unani and Traditional systems are the main systems of indigenous medicines. Researchers are providing evidence through research, in validating the efficacy and safety indigenous drugs.

The selected plant *Salacia reticulata* plays a considerable role in Sri Lankan folk medicines as treatment to Urolithiasis. In addition to the kidney stone disease, treatments of diabetes, rheumatism, gonorrhoea and other skin diseases are prepared by using *Salacia reticulata* which is known to provide safe and well- tolerated remedies for chronic illnesses which typically resulted from the combinations of secondary plant metabolites that are synthesized and deposited in specific parts of the plant. *Salacia reticulata* is an indigenous flowering plant grown in dry zone forests in Sri Lanka. In Ayurvedic medicine, it is known as *Kothalahimbutu* in Sinhala and *Karanthi, Ponkoranthi* in Tamil. It is a climbing, perennial, woody shrub which has a dichotomous branching pattern. The bark of this plant is a thin smooth one with grey colour outside and white colour internally. The leaves are opposite and elliptic oblong. Its flowers are bisexual and they are arranged as 2-8 clusters in leaf axils. The fruit is a drupe which is globose and tubular. The extract from the plant has been used for the treatment of urolithiasis, diabetes, rheumatism and gonorrhoea in Ayurveda [10].

This study was aimed to evaluate In-vitro anti-urolithiatic activity of methanolic extract of *Salacia reticulata* on experimentally prepared calcium oxalate stones and to compare it with the in-vitro anti- urolithiatic activity of the standard formulation.

Materials and methods

Extraction process

Plant *Salacia reticulata* was collected from Hettipola area in Kurunegala, Sri Lanka (Figure 1). The plant material was taxonomically identified and authenticated by the taxonomist of Department of Botany, University of Jaffna, Sri Lanka. Finely cut stem parts of the plant *Salacia reticulata* were shade

dried at room temperature and finely pulverized by using blender. Methanolic extract was obtained by using 200g of powdered stem and using 400ml of methanol (analytical grade, Merk specialties private Limited) in soxhlet apparatus until all the compounds were extracted in to the solvent. The extract was concentrated by using rotary evaporator at 40^0C.

Figure 1: Plant *Salacia reticulata*

Preparation of calcium oxalate crystals by homogeneous sprecipitation method

$$CaCl_2 + Na_2C_2O_4 \longrightarrow CaC_2O_4 + 2NaCl$$

Calcium Chloride dihydrate (4.41g, analytical grade, Park Scientific Limited) dissolved in distilled water and Sodium Oxalate (4.02g, analytical grade, Research – Fine Chem Industries) dissolved in 2N Sulphuric acid were taken in two separate beakers and both solutions were mixed together with stirring until Calcium oxalate precipitate formed. Excess Sulphuric acid was removed by washing with Ammonia solution (analytical grade, Merk specialities private Limited) and distilled water respectively and allowed to dry at 60°C for 4 hours.

Preparation of semi-permeable membrane from chicken eggs

Semi permeable membrane was prepared from chicken egg by decalcifying outer shell and removing inner contents of egg. Apex of egg was punctured by a glass rod to remove the entire content. Empty egg shells were washed thoroughly with distilled water and placed in a beaker consisting 2M HCl (analytical grade, techno pharmache) for an overnight which caused complete decalcification. Then membranes were washed with distilled water and placed in ammonia solution for neutralization in the moistened condition for a while. Then they were rinsed with distilled water and Stored in a refrigerator at a pH of 7- 7.4[11]. Preparation of semi-permeable membrane and prepared membranes are shown in Figure 2.

Figure 2: Preparation of semi-permeable membrane and prepared membranes

Evaluation of anti-urolithiatic activity by the titrimetric method

The dissolution percentage of Calcium Oxalate was evaluated as described below by using titrimetric method with different three groups (Table 1).

Table 1: The dissolution percentage of Calcium Oxalate was evaluated as described below by using titrimetric method

Group	Concentration
Negative control	5 mg Calcium Oxalate
Positive control	5 mg Calcium Oxalate + 10, 20, 30, 40 mg of Cystone
Salacia reticulata.	5mg Calcium Oxalate + 10, 20, 30 and 40 mg of *Salacia reticulata*

Each sample was packed in separate semi permeable membranes and mixed well. Then semi permeable membranes were allowed to suspend in separate conical flasks which contained 100mL of the buffer solution. Conical Flasks containing the samples of all groups were kept in an incubator at 37°C for 7 hours. The contents of the semi permeable membranes from each group were removed into separate test tubes. A 5mL of 1 N Sulphuric acid was added to each test tube and the resultant mixture was titrated with 0.01 N KMnO4 until a light pink colour end point is obtained.

Calcium oxalate is reacted with the extract/standard and get dissolved based on the ability of test substances to dissolve it in a semipermeable membrane which is suspend in a buffer at pH 7.4. The undissolved Calcium oxalate remaining inside the semipermeable membrane is estimated in acidic condition by titrating against standarized KMnO4, purified from Merk specialities Private Limited. The percentage of dissolution of Calcium oxalate is calculated using the insoluble calcium oxalate.

Each 1 mL of 0.01N KMnO4 is equivalent to 0.64 mg of Calcium oxalate.

Amount of Calcium Oxalate in dissolution sac in the beginning = 5mg

Estimation of Calcium Oxalate in dissolution sac after incubation = (VKMnO4 × 0.64)

Reduced Calcium Oxalate amount

= 5mg - (VKMnO4 × 0.64)

Dissolution Percentage

= (Reduced Calcium Oxalate)/5mg ×100

Total quantity of dissolved Calcium Oxalate weight was obtained by subtracting the remaining insoluble Calcium Oxalate from the total quantity used in the beginning of the experiment[12].

Results and Discussion

Yield percentage of methanolic extract of Salacia reticulata

Yield percentage of methanolic extract of Salacia reticulata is shown in Table 2.

The mean (±SD) dissolution percentages of methanolic extract of Salacia reticulata on experimentally prepared Calcium Oxalate for 10, 20, 30 and 40mg concentration were 34.8%, 38.6%, 40.4% and 45.0% respectively.

Table 2: Yield percentage of methanolic extract of Salacia reticulata

Initial weight	Crude weight	Yield percentage	Colour of the crude product
100g	15.50	15.5%	Dark brown

The mean (±SD) dissolution percentages of standard drug Cystone, Himalaya company, India on experimentally prepared Calcium Oxalate for 10, 20, 30 and 40mg concentration were 64.8%, 70.6%, 75.8.%, 79.6% respectively (Figure 3 and Table 3).

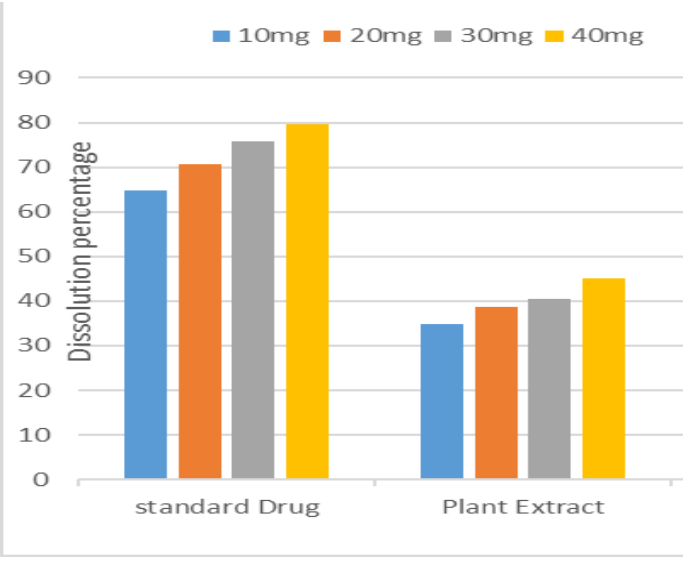

Figure 2: Dissolution percentages of Calcium Oxalate by the standard and the selected plant extract

Table 3: Dissolution percentage of Calcium oxalate by plant extract and the standard

Groups	Concentrations	Volume of $KMnO_4$*(ml)	Weight of Calcium oxalate estimated** (mg)	Weight of Calcium oxalate reduced*** (mg)	Dissolution percentage (%)
Negative control	5mg	05.20	3.33	1.67	33.4
Standard	10mg	02.75	1.76	3.24	64.8
	20mg	02.30	1.47	3.53	70.6
	30mg	1.89	1.21	3.79	75.8
	40mg	1.60	1.02	3.98	79.6
Salacia reticulata	10mg	05.10	3.26	1.74	34.8
	20mg	04.80	3.07	1.93	38.6
	30mg	04.65	2.98	2.02	40.4
	40mg	04.30	2.75	2.25	45.0

*After the incubation remaining calcium oxalate in the sac was titrated with $KMnO_4$.

**Weight of calcium oxalate estimated by titration with $KMnO_4$

***Weight of calcium oxalate reduced due to the dissolution by plant extracts and the standard.

The study could be proceeded by using stones removed from the patients who affected by the kidney stones with aqueous extract.

Conclusion

Titration method was used in the study to evaluate the anti- urolithiatic activity of *Salacia reticulata* against the experimentally prepared calcium oxalate stones. The mean dissolution percentages of methanolic extract of *Salacia reticulata* on experimentally prepared calcium oxalate at 10, 20, 30 and 40 mg were 34.8%, 38.6%' 40.4% and 45.0% respectively. The dissolution percentage was significantly increased with concentration. There was a significant correlation between dissolution percentage and concentration. Dissolution percentages for the standard drug against calcium oxalate stones in the given concentration were 64.8%, 70.6%, 75.8% and 79.6% respectively. Both the methanolic extract of the plant and the standard drug have a positive correlation with the concentration. The results of this study have confirmed the long history of the use of *Salacia reticulata* in traditional medicine for the treatment of Urolithiasis.

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In Vitro Evaluation and Comparison of Antibacterial Activity of the Novel Herbal Aqueous Creams Containing Aqueous Extracts of the Leaves of *Ficus religiosa* L.

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Abstract

In the current healthcare system, antibiotic resistance has become one of the crucial health issues. Discovering a novel antibiotic is a very complex and time-consuming process. Hence there is an increasing trend of conducting researches using herbal sources to treat infections which are resistant to currently available antibiotics. The objective of this study was to in vitro evaluation and comparison of the antibacterial property of herbal creams containing aqueous extracts of leaves of *Ficus religiosa* L. against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Ficus religiosa* L. is a plant which has been using for many decades in Ayurvedic medicines to treat various types of illnesses including dermatological diseases. In this study, the antibacterial effect of the leaf extract was evaluated using agar well diffusion method. Using a dose-response curve, different concentrations of the extract were selected to formulate herbal creams. According to British Pharmacopoeia 2015, the aqueous cream samples were formulated without adding the preservative. Antibacterial effects of creams were evaluated against *S.aureus* and *P. aeruginosa.* The plant extract has shown a promising inhibitory action against the tested organisms. Some higher concentrations of plant extracts have shown a higher Zone of Inhibition when compared with the positive control (Gentamicin 50 µg/mL). All the cream samples have shown more inhibitory action against *S. aureus* than *P. aeruginosa.* The stability of the cream samples was evaluated. No change was observed in appearance, color and odor of cream samples in the period of 3 months. The overall results of this study indicate that the herbal creams have promising antibacterial properties and these results could be utilized in the pharmaceuticals industry to carry out more testing procedures to develop as an efficient antibacterial cream.

Keywords: Antibacterial property, Antibiotic resistance, *Ficus religiosa* L., Skin diseases, Formulations, Herbal creams

Introduction

The skin is considered as the largest organ in the human body. It is persistently exposed to potentially hazardous microbial and non-microbial agents of the environment. It acts as a physical barrier and provides the first lines of defense against these agents[1]. When treating dermatological diseases such as skin inflammations, microbial and fungal skin infections and skin cancer, the skin is considered as the main route of choice[2,3].

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Figure 1: Tree of *Ficus religiosa* L

Ficus religiosa (Figure 1) belongs to the family "Moraceae". It *is* a medicinal plant which is commonly used in Ayurvedic and other traditional medical systems. It is known as the "Peepal" tree or "sacred fig" in English whereas in Sinhala it is known as the "Bo" tree. A wide range of diseases such as bacterial infections, viral infections, diabetes, helminthic diseases, dyslipidemia and cancers are treated using different parts of *F. religiosa* tree[4,5,6]. Various studies have been conducted to evaluate the antibacterial, antioxidant, antidiabetic, antiulcer, antiarthritic and anti-inflammatory effects of the different parts of the *F. religiosa* tree[6,7,8]. It is also used to treat various types of diseases of the gastro intestinal tract (vomiting, stomatitis, constipation, liver diseases), central nervous system (migraine, epilepsy), respiratory system (asthma, cough), infectious diseases (leprosy, tuberculosis, gonorrhea) and reproductive system. As well as the leaves and barks of *F. religiosa* are used to treat skin diseases[14,15,16].

Antibiotic treatment kills or suppresses the growth of a significant part of the microbial population by various mechanisms of action[11]. The effectiveness of presently available antibiotics is reducing significantly due to the emergence of resistance. According to World Health Organization (WHO), antimicrobial resistance is among one of the millennium developments goals at risk and endangers the achievement of sustainable development goals[12]. It is one of the greatest necessities to discover or develop novel antibiotics from natural/herbal sources to treat infections which are resistant to currently available antibiotics[13].

F. religiosa trees can be commonly found in the sub-Himalayan forests in Bengal and central India as well as in countries which are located in the Indian sub-continent like Pakistan, Bangladesh and Sri Lanka[17,14]. In Ayurveda, "*Panchavalkala*" is known as a combination of five barks of trees namely; *Nyagrodha* (*F. benghalensis* L.), *Udumbara* (*Ficus racemosa* Linn.), *Ashwatha* (*F. religiosa* L.), *Parisha* (*Thespesia populnea*) and *Plaksha* (*Ficus lacor*). This combination is widely used in Ayurveda as anthelmintic, antimicrobial, wound healing, anti-inflammatory agent and it is also used for cleansing purposes[18,19].

Many studies have been conducted to evaluate the antibacterial properties of *F. religiosa*, especially in India. An only a limited number of antibacterial preparations were formulated using this plant. The object of the present study was to *in vitro* evaluate, formulate and compare the antibacterial activity of herbal creams containing aqueous extract of *F. religiosa* leaves against *S. aureus* and *P. aeruginosa.*

Materials and Methods

Collection and preparation of plant material

The leaves were washed from distilled water to remove dust, soil and other materials on the surface of leaves and air-dried under shade for three weeks. Dried leaves were ground into powder by using an electric grinder. Powdered materials were stored in an airtight container for further use at room temperature (25 ± 5°C).

Preparation of aqueous extracts

Powdered leaves (50g) was added to a beaker containing 250 mL of distilled water. Each powder sample was kept on a mechanical shaker (Fisherbrand™ Seastar™ Digital Orbital Shaker) at 200 rpm and macerated for 48 hours, at room temperature. Then the macerates were filtered through two layers of clean muslin cloth in order to remove debris. The remaining aqueous portion was

removed using the freeze dryer (LABCONCO FreeZone 2.5). The extracts were labelled and stored in a refrigerator at 4-8°C until further use[20].

Preparation of concentration series for the microbial assay

A serial dilution was prepared using the final filtrate which started from 2000 µg/mL to 125 µg/mL.

Preparation of stock solution of Gentamicin as positive control

The stock solution was prepared using a commercially available Gentamicin (40 mg/mL) IV injection vial. 125 µL of Gentamicin solution was measured using a micropipette and transferred into a 100 mL volumetric flask. Then the flask was topped up with autoclaved distilled water until it reached the final volume of 100 mL.

Screening antibacterial effect of extracts of the leaves

Pure cultures of *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 25923) were obtained and sub-cultured in nutrient agar and stored in the refrigerator (4-8°C). The 0.5 McFarland standard was prepared by mixing 0.5 mL of 1.175% W/V $BaCl_2$ and 99.5 mL of 1% V/V H_2SO_4 with constant stirring to maintain a suspension.

Muller Hinton Agar (MHA) was prepared and after measuring the pH of the prepared agar solutions, they were placed in the autoclave at 121°C at 15 pounds per square inch for 15 minutes.

According to the direct colony suspension method, bacterial colonies of each organism (*S. aureus* and *P. aeruginosa*) were added separately to two sterile test tubes filled with 5 mL of saline solutions using a sterile inoculating loop. A single colony was added to saline solutions until turbidity is equivalent to the previously prepared 0.5 McFarland solution. These bacterial suspensions were used within 15 minutes of preparation.

Using separate micropipette tips per each bacteria, 1 mL of previously prepared bacterial suspensions were added into each MHA containing flask at about 40-45°C. Bacteria containing MHA solutions were added into autoclaved petri dishes using the pour plate method and six wells with equal diameter were prepared for each petri dish. Each well was filled by 200 µL of serial dilutions of the plant extract, Gentamicin 50 µg/mL (positive control) and distilled water (negative control). The plates were incubated at 37°C for 24 hours. After the 24 hours incubation period, the zones of inhibition around the wells were measured to the nearest millimeter and recorded.

Formulation of antibacterial herbal aqueous creams

According to the results of the antimicrobial assay of plant extracts, dose response curves were plotted using GraphPad Prism 6 software (version 6.01). Using the dose response curves, four concentrations of aqueous extract of *F. religiosa* were selected to formulate cream preparations.

As per BP 2015, emulsifying wax and Emulsifying ointment were prepared. The powdered extract was incorporated together with Emulsifying ointment, without adding the preservative (Phenoxyethanol) to prepare aqueous cream as described in BP.

Antibacterial assay of prepared herbal aqueous creams

Antibacterial assay of prepared herbal aqueous creams was carried out according to BP method of Gentamicin cream assay.

Microbial assay of cream samples

MHA medium was prepared and added test organisms were into MHA solutions. The mixtures were added into petri dishes using the pour plate method. In each petri dish, wells were prepared with equal diameter. Each well was filled by 200 µL of a solution obtained from the aqueous layer (top layer) of the separatory funnel containing herbal aqueous cream, Gentamicin (positive control) and the solution obtained from the aqueous layer (top layer) of the separatory.

Results

The results of the antimicrobial effects of leaves extract against *P. aeruginosa* are shown in Table 1.

Table 1: Zone of Inhibition shown by *Pseudomonas aeruginosa* with crude extracts of leaves

Concentration (µg/mL)	ZOI (mm)
2000	18.67±0.33
1750	18.50±0.40
1500	18.33±0.67
1250	18.00±0.00
1000	17.00±0.58
750	16.50±0.40
500	12.67±0.88
250	11.67±0.33
125	11.30±0.33
Gentamicin (50 µg/mL)	19±0.00
Distilled water	00.00

Data in the above table was expressed as mean Zone of Inhibition (mm) ± SEM.

According to the data expressed in Table 1, the positive control (Gentamicin 50 µg/mL) has shown a very clear ZOI compared to the ZOI which was shown by all plant extracts.

Antibacterial effect of leaves extracts against Staphylococcus aureus

The results of the antimicrobial effects of leaves extract against *S. aureus* are shown in Table 2.

According to the results expressed in Table 2, higher ZOI has exhibited against *S. aureus* by the aqueous extract of *F. religiosa* 1500 µg/mL, 1750 µg/mL and 2000 µg/mL concentrations when compared with the ZOI shown by Gentamicin 50 µg/mL against *S. aureus.*

Table 2: Zone of Inhibition shown by *Staphylococcus aureus* with crude extracts of leaves

Concentration (µg/mL)	ZOI (mm)
2000	18.33±0.67
1750	17.33±0.88
1500	17.00±0.00
1250	15.67±0.67
1000	12.67±0.88
750	12.33±0.88
500	11.50±0.40
250	11.30±0.67
125	11.00±0.00
Gentamicin (50 µg/mL)	15±0.00
Distilled water	0.00

Data in the above table was expressed as mean Zone of Inhibition (mm) ± SEM.

Dose-response study

Dose-response curves of aqueous leaves extract of *F. religiosa* against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Figure 2).

According to the dose-response curve in Figure 2, four concentrations of aqueous leaves extracts of *F. religiosa* were selected to formulate the herbal creams and they were labelled as follows (Table 3 and Figure 3 and Figure 4).

Table 3: Selected concentrations of aqueous leaves extracts of *Ficus religiosa* to formulate herbal aqueous creams

Concentration of crude extract (µg/mL)	Labelled as
500	FR aq 1
800	FR aq 2
1000	FR aq 3
1800	FR aq 4

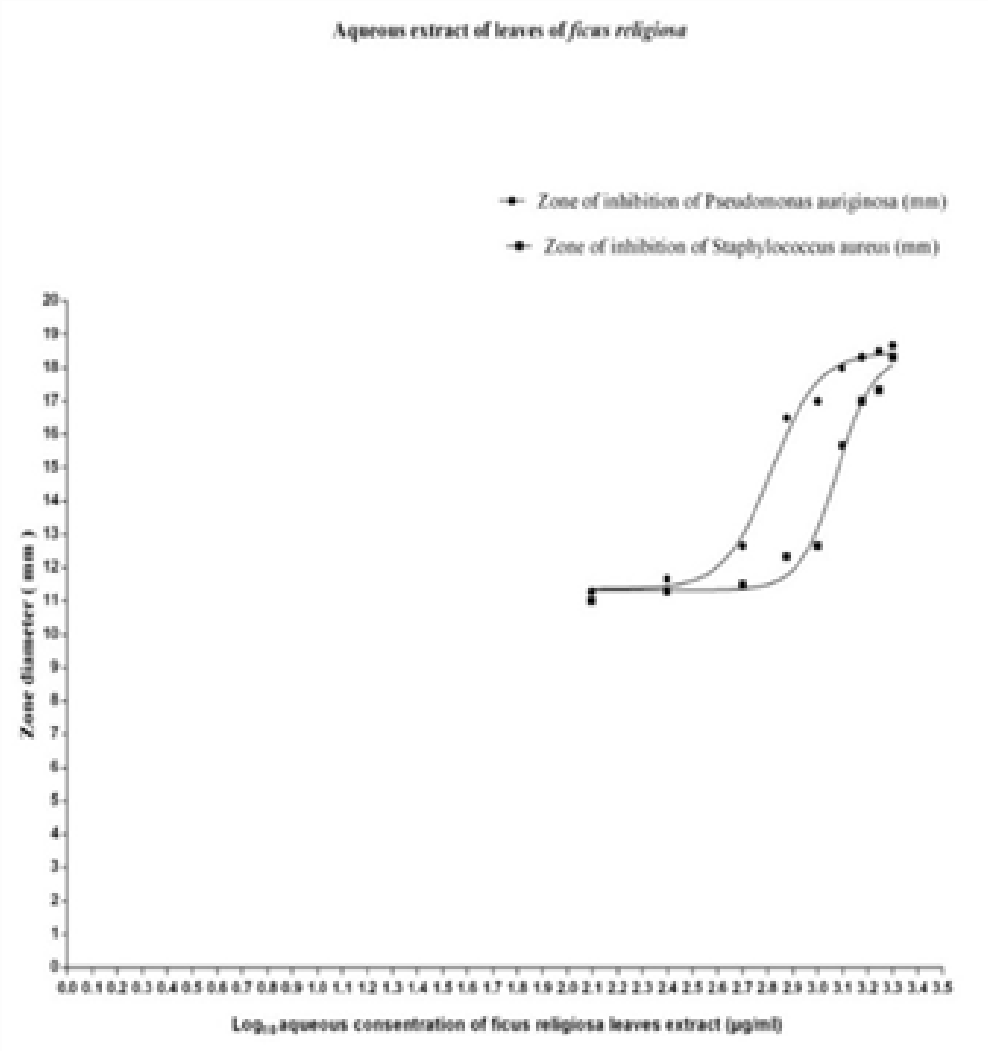

Figure 2: Dose response curve of aqueous leaves extracts of *Ficus religiosa* against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

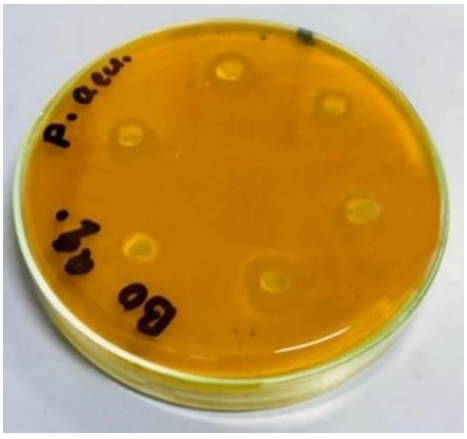

Figure 3: Antibacterial effect of aqueous extract of *Ficus religiosa*, against *Pseudomonas aeruginosa*

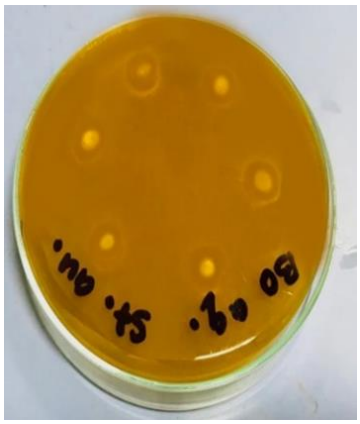

Figure 4: Antibacterial effect of aqueous extract of *Ficus religiosa*, against *Staphylococcus aureus*

Table 4 and Figure 5 and Figure 6 shows antibacterial effect of herbal aqueous cream containing aqueous extract of *Ficus religiosa* leaves against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Table 4: Antibacterial effect of herbal aqueous cream containing aqueous extract of *Ficus religiosa* leaves against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Sample	Zone of Inhibition (mm)	
	Staphylococcus aureus	***Pseudomonas aeruginosa***
FR aq 1	11.00±0.00	10.67±0.33
FR aq 2	12.00±0.00	10.67±0.33
FR aq 3	12.33±0.33	12.00±0.00
FR aq 4	14.00±0.58	12.00±0.58
Gentamicin (50 µg/mL)	19.00±0.00	15.00±0.00
Distilled water	0.00	0.00

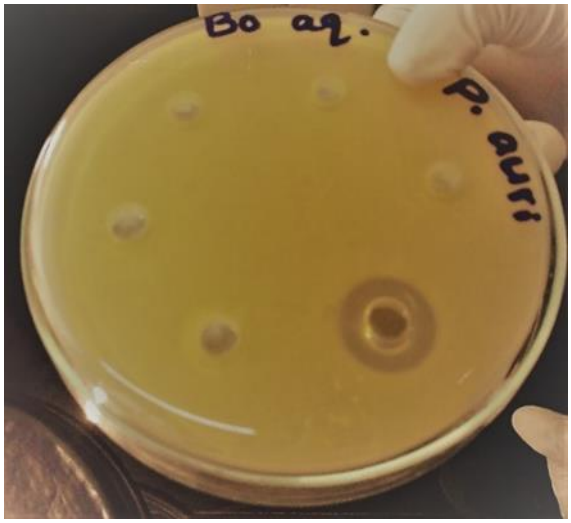

Figure 5: Antibacterial effect of herbal aqueous cream containing aqueous extract of *Ficus religiosa*, against *Pseudomonas aeruginosa*

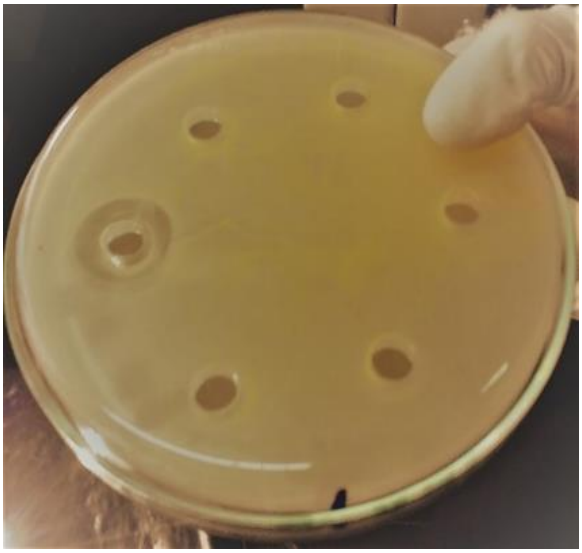

Figure 6: Antibacterial effect of herbal aqueous cream containing aqueous extract of *Ficus religiosa*, against *Staphylococcus aureus*

Stability evaluation

Evaluation of short-term stability

According to figure 7, four cream samples containing aqueous extracts *F. religiosa* were stable at room temperature throughout the study period of 16 days. Creaming, phase separation or coalescence couldn't be observed throughout the study period of 16 days.

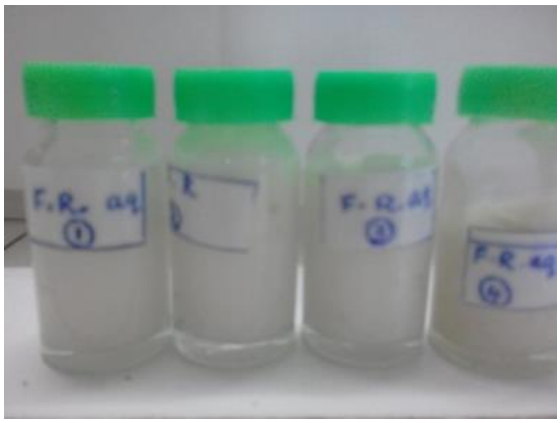

Figure 7: Aqueous herbal creams containing aqueous extracts of *Ficus religiosa* on the 16th day from the date of formulation

Figure 8 shows the aqueous herbal creams containing aqueous extracts of *Ficus religiosa* on the 90th day from the date of formulation

Figure 8: Aqueous herbal creams containing aqueous extracts of Ficus religiosa on the 90th day from the date of formulation

Evaluation of accelerated stability

Upon centrifugation of prepared herbal aqueous creams, the entire cream samples were stable throughout the period of 14 days (Figure 9).

Figure 9: On the 14th day after centrifugation aqueous herbal creams containing aqueous extracts of *Ficus religiosa*

Characterization of creams

pH evaluation

The pH values of all the cream preparations were within the range of 5.21-5.32 (Table 5) which are compatible with the pH range that should be included in a dermatological preparation (pH 4.5-6.0)22. No significant changes in pH were observed during the period of testing.

Viscosity

Viscosity of the cream samples at 28.2 ºC shown in Table 6.

Microscopic analysis

According to figure 10, herbal aqueous creams contain O/W emulsion.

Table 5: pH values of herbal cream samples containing aqueous extracts of *Ficus religiosa*

Type of cream	pH value					
	1st	15th	30th	60th	75th	90th
FR aq 1	5.21	5.23	5.19	5.20	5.22	5.22
FR aq 2	5.23	5.25	5.24	5.24	5.23	5.24
FR aq 3	5.22	5.24	5.23	5.23	5.25	5.23
FR aq 4	5.26	5.24	5.25	5.26	5.24	5.25

Table 6: Viscosity of the cream samples at 28.2 ºC

Sample	Viscosity (cps) at rpm					
	0.3	0.6	1.5	3	6	12
FR aq 1	360,000	194,000	97570	65786	43590	31443
FR aq 2	359,700	192,580	97345	63580	42670	31170
FR aq 3	361,245	193,585	97438	63650	42680	31357
FR aq 4	359,860	193,000	97447	63570	42785	31379

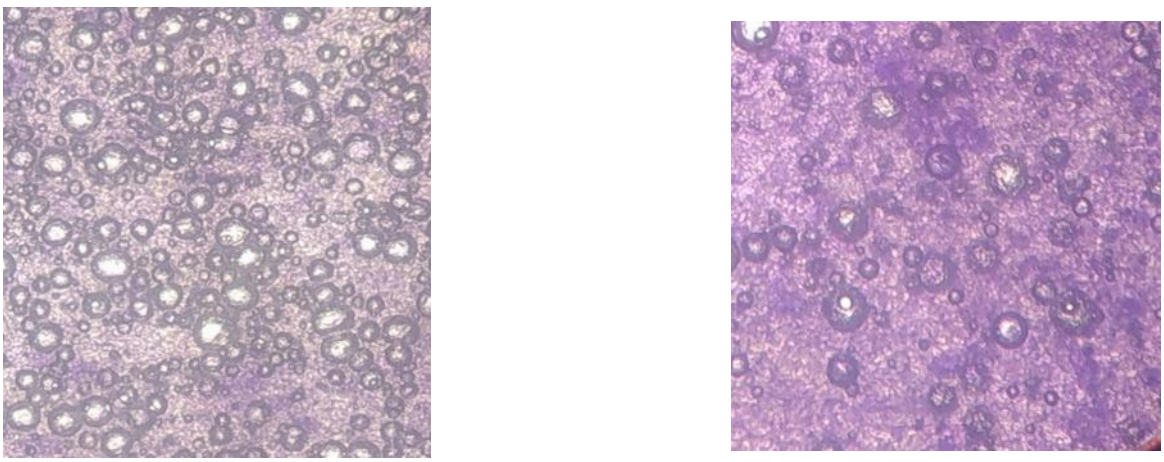

Figure 10: Microscope analysis of aqueous herbal creams containing aqueous extracts of *Ficus religiosa* under 100X magnification

Discussion

Creams have been used for many centuries to improve the wound healing process, to treat dermatological diseases, to protect the skin, as well as to improve natural beauty. Creams are one of the most popular dermatological preparations available, because of its semisolid properties facilitate ease of application, ease of removal and better penetration of drug/s into the skin layers[23,24].

Aqueous cream BP is a widely prescribed product as an emollient and soap substitute in the treatment of dry skin conditions. It relieves skin dryness by providing moisture to the skin. The Medicines and Healthcare products Regulatory Agency (MHRA) in the United Kingdom indicates that Sodium Lauryl Sulphate contained in aqueous cream is mainly responsible for stabilizing the O/W emulsion rather than its detergent property.

Dermatological diseases are one of the major health conditions in developing countries. The majority of cutaneous bacterial infections are caused by either *Staphylococcus aureus* or *Streptococci*[25,9].

In the current healthcare sector, antibiotic resistance has become one of the most critical health issues facing patients and healthcare workers[12]. Discovering a new therapeutic agent is a complex process and it consumes more than 10 years to reach a drug to the market[26]. However, many researches are being conducted to discover or develop novel antibiotics from herbal sources to treat infections which are resistant to currently available antibiotics[13].

Since ancient times, plants have been used to treat a vast range of diseases. At the present, there is an increasing trend to utilize herbal medicines and in most of the developing countries, people tend to use herbal medicines because of better compatibility with the body, lesser side effects and better cultural acceptability[26,27,28].

In the current study, *in vitro* antibacterial properties of aqueous extracts of *F. religiosa* were evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* separately. Moreover, using selected concentrations of this crude substance, aqueous herbal creams were formulated and evaluated for their antibacterial properties. This plant was selected to conduct this study because it is still used in Ayurvedic medicine in Sri Lanka. As well as a collection of plant materials is unchallenging because they are highly abundant in the area.

Before conducting the research, plant materials were authenticated by the National Herbarium, Peradeniya, Sri Lanka. It is one of the crucial steps to identify the plant materials prior to the study because many plants that belong to the same family are similar in appearance and it may cause misleading the results of the study.

Even though in Ayurveda medicine, barks of the plant are used to obtain the antimicrobial property, according to literature leaves of *F. religiosa* L. have been proven to possess more antibacterial activity than that of the barks of the tree[29,16]. Hence leaves were used in the current study to evaluate the antibacterial property.

In this study, the maceration process was used to obtain the aqueous plant extract. In order to increase contact between plant materials and the solvent, dried leaves were ground using a grinder. A mechanical shaker was used during the maceration process to increase the speed of the maceration.

In this research, the antibacterial effect of the crude plant extract and the herbal aqueous creams containing the plant extract was evaluated against two organisms. *Pseudomonas aeruginosa* is a Gram-negative bacteria while *Staphylococcus aureus* is a Gram-positive bacteria. These two kinds of bacteria were used in this study to identify and compare the inhibitory activity of *F. religiosa* against both kinds of bacteria.

According to the literature, *F. religiosa* leaves extracts have shown antibacterial action against both Gram-positive and Gram-negative bacteria[16,20,6]. Considering table 1 and 2, it has also been proven in this study. It was noted that aqueous extract has shown more inhibitory action against *P. aeruginosa* than *S. aureus.* The same result was obtained in the study carried by Shrijana *et al*., 2017.

Gentamicin (50 µg/mL) was used as the positive control in this study and it has shown 19 mm and 15

mm of ZOI against *P. aeruginosa* than *S. aureus* respectively.

According to table 2, 1250 µg/mL, 1500 µg/mL, 1750 µg/mL and 2000 µg/mL of aqueous extracts of *F. religiosa*, have shown the same or greater ZOI against *S. aureus* when compared to Gentamicin (50 µg/mL).

According to the literature, the majority of cutaneous bacterial infections are caused by *S. aureus* than *P. aeruginosa*[25,9]. Hence according to table 2, aqueous extract of *F. religiosa* has promising activity against skin infections caused by *S. aureus* when compared with Gentamicin (50 µg/mL).

Using the linear range of the dose-response curve of crude plant extract, suitable concentrations were selected to formulate the cream samples. The concentrations of the plant extract which were selected from the linear range of the dose-response curve were incorporated into the aqueous phase of the cream samples.

The herbal creams were formulated using the fusion method (Pharmalabs.unc.edu, 2017). The literature reveals that constituents in the leaves of the plant can be heated around 100°C without destroying the properties of the crude substances[26,16]. It is recommended to heat the aqueous phase a few degrees higher than the oil phase when both phases are being mixed to make a stable ointment because the aqueous phase tends to cool faster than the oil phase. This may lead to solidification of the active ingredient or excipients and finally, phase separation may occur[30].

In this study, the preservative of aqueous cream BP (Phenoxyethanol) was not included when preparing the herbal cream samples assuming it may have an impact on the result of the antibacterial effect of formulated herbal cream samples[31].

Prior to conduct the microbial assay of the prepared cream samples, plant extracts were separated according to the BP 2015 method of Gentamicin cream assay. In this study, only the aqueous layer inside the separatory funnel was used to evaluate the inhibitory action of the cream samples with the assumption of crude extracts may retain in the aqueous phase of the herbal creams without diffusing them to the oil phase.

According to tables 3, all tested concentrations of all the prepared herbal cream samples containing leaves extracts have shown less ZOI against both organisms when compared to the positive control (Gentamicin (50 µg/mL). The concentrations of each cream sample have diluted than the selected concentrations from the dose-response curves because the calculations were made to formulate the creams as per the selected concentrations were included in the aqueous phase of the creams. As well as during the separation procedure which was done prior to the assay of the creams, some amount of the crude extracts in the aqueous phase might be diffused to the oil phase. Considering the results in table 4, almost all the herbal creams have shown more inhibitory action against *S. aureus* than that of *P. aeruginosa*.

Stability testing of a drug product is crucial, in order to maintain the quality and safety and to determine the shelf life of the product. A formulation may undergo changes like its pH, consistency and appearance. Mainly there are two types of stability testing based on the time duration of stability evaluation namely; accelerated stability testing and real-time stability testing (Bhagwat *et al*., 2017). In this research, the samples were undergone stress conditions to evaluate the accelerated stability such as high temperature (37°C), low temperature (2-8°C) for four weeks and centrifugation.

No significant changes in color or odor were observed in each cream sample which was stored at room temperature, at 37-38°C and in the refrigerator (2-8°C) for 90 days.

The pH of the freshly prepared creams was measured in this study. The pH values of all the cream preparations were within the range of 5.21-5.32 (Tables 5) which are compatible with the pH range that should be included in a dermatological preparation (pH 4.5-6.0)[22].

The main purpose of this study was to formulate antibacterial creams using aqueous extracts of the leaves of *F. religiosa* L. compare the antibacterial activity of these formulations against tested

organisms and determine the stability of prepared herbal cream samples. The overall results of this study indicate that the herbal aqueous creams containing aqueous extract of the leaves of *F. religiosa* have promising antibacterial properties and these results could be utilized in the pharmaceuticals industry to carry out more testing procedures to develop as an efficient antibacterial cream.

Conclusion

The results of the present study suggest that selected plants can be used to treat various types of bacterial infections. According to the results obtained from the evaluation of the antibacterial effect of cream samples, it can be concluded that all cream samples have promising antibacterial activity against skin infections caused by *S. aureus* when compared with the skin infections caused by *P. aeruginosa.*

Recommendations

The present study indicates that the herbal aqueous creams containing aqueous extracts of the leaves of *F. religiosa* L. when further developed, can be used as effective dermal preparation for bacterial skin infections. The most active molecules can be identified and isolated by conducting a further pharmacological evaluation. As well as the safety and the activity of these cream samples can be further evaluated by conducting clinical trials.

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Competing Ideologies and Reforms in Traditional Medicine from 1948-1960

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Abstract

Those who involved in reforming Traditional Medicine in Sri Lanka believed that informally trained (*Deshiya Chikithsa*) practitioners and Ayurveda practitioners were able to create a common platform to reform if after independence. Nevertheless, traditional medicine became a highly contested phenomenon in the aftermath of independence from 1948-1960. The objective of this study was to study how the reforms proposed by the so-called progressive front led by Dr. Lenora and others became highly controversial issues between 1948 and 1960. This research project was carried out as a qualitative study at various institutes in the United Kingdom and Sri Lanka. The researcher of this study used a digital camera to film all relevant material at various institutes and organized them into logical themes and analysed them according to theme-list and content analysis methods. This study findings reveal that the existing ideologies between the two opposing groups discouraged them to reach a common platform bring about syntheses to safeguard the Ayurveda system of Medicine and *Deshiya Chikithsa* for the betterment of the country. Therefore, the focus of reforms was, therefore, what can be referred to as the 'biomedicalization' of TM; an approach that continued to stoke tensions among politicians, administrators, and TM practitioners. Therefore, these research findings speak the need of conducting research to identify ways to create harmonious grounds to bridge Ayurveda and *Deshiya Chikithsa* with modern scientific methods without losing the authenticity of the two for the benefit of the people of Sri Lanka.

Keywords: Ayurveda, *Deshiya Chikitsa*, reforms, practitioners, progressive

Introduction

Although Since the beginning of British reforms in traditional medicine in Sri Lanka/Ceylon, there have been conflict-ridden, controversial and contested debates on the subject among various groups as to how such reforms should be carried out to develop people's health in the country. These study findings reveal that, after independence the reforms in traditional medicine in Sri Lanka became highly contested field with the growing Sinhalese Buddhist Nationalism.

The main reason was that its discourse demanded a reaffirmation of lost cultural identify during more than four hundred years of colonial rule in the country. These debates embroiled with ongoing nationalist politics of the Sinhala Buddhist movement led by Bandaranayake and other organizations sphere headed by more urban-based liberal intellectuals and some of whom had training in western and traditional medicine.

This way the anti-modernist group was led by a group of recent graduates of the Colleges of Indigenous medicine and Gampaha Siddhayurveda College, members of the Ceylon Ayurveda Congress, informally trained traditional medical practitioners and other nationalist leaders led by Pundith G.P.Wickramarachchi. The modernist group was led by the Venerable Malewana Gnanissara and Dr. L.B. Lenora who was the then principal of the College of Indigenous medicine. They were supported by groups of teachers and graduates of the College of Indigenous Medicine and Gampaha Siddhayurveda College and the then the Minister of Health E.A. Nugawela. The purpose of this article is to discuss how all the attempts that the so called progressive front utilised the silver jubilee of the

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college of Indigenous medicine-related activities and the Lenora System to introduce reforms to develop traditional medicine failed due to unprecedented opposition from the anti-reformist group in the 1950s in Sri Lanka.

Methodology

This research project was carried in the United Kingdom and Sri Lanka as a part of the author's doctoral study that was submitted to the University of London. Therefore, the material in this research paper originated from this research and other secondary sources. The data for this study was collected at various research organizations and institutes located both in the United Kingdom and Sri Lanka. These institutions ranged from the British National Archives, the British National Library, the School of African and Oriental Studies, the Welcome Centre Library, the Sri Lanka National Archives, the Bandaranayake Memorial Ayurveda Research Institute at Maharagama, Sri Lanka, the Sri Lanka Museum Library, the Library of the Institute of Indigenous Medicine, University of Colombo Sri Lanka and to the Library of the University of Peradeniya, Sri Lanka.

After obtaining legal permission, the researcher spent around two years collecting data at the above-mentioned places in the two countries. For this, the researcher was legally allowed to use a digital camera to film all relevant primary research material. Subsequently, all filmed material was stored into a computer and then printed thousands of pages at the Welcome Centre Printing Unit which was by then affiliated to the University of London. In addition, the researcher had the privilege to use relevant sources from a number of individual collectors in Sri Lanka. The whole study was conducted as a qualitative research project. The collected data was organized into relevant themes and analysed them using two qualitative techniques known as theme-list and content analysis methods.

The silver Jubilee of the College of Indigenous Medicine, and Traditional Medicine Reforms in 1954

Amidst growing opposition to Traditional Medicine (TM) reforms, the Board and the College of Indigenous Medicine organized a public ceremony to commemorate the twenty-fifth anniversary of the College in 1954 [1]. The organizing committee arranged a weeklong commemoration ceremony, which included exhibitions, academic sessions, discussions, and Western and TM-based clinics, with state support, from 05th-11th January 1955. As part of the celebration, the organizing committee published a souvenir incorporating statements from prominent figures in the government and in the field of TM. In the commemorative volume, the following dignitaries of the government and the opposition issued statements: S.W.R.D Bandaranayake (Leader of the Opposition), Sir John Kothalawala (Prime Minister), Sir Oliver Gunathilake (Governor of Ceylon), and E.A. Nugawela (Minister of Health) [2]. The following excerpts are taken from the jubilee volume:

"*The Government has recognised and given state aid to the indigenous system of medicine starting in the year 1928 with a grant of RS.75, 000. Today the College and Hospital of Indigenous Medicine are maintained as Government Institutions at an expenditure in the neighbourhood of Rs.1, 000,000. The College has turned out over five hundred trained practitioners of indigenous medicine and the Outpatients' Department of the Hospital is now treating about one thousand a day* (*Sri Oliver Gunathilake, Governor General of Ceylon*)"[3].

"*The 25th anniversary of the Government Ayurvedic Hospital and Vidyalayais of great importance in the development of the Island's health services. This institution has been more than a mere hospital and*

medical school, for it has helped in obtaining for Ayurveda an important place in the health services of the nation. This would not have been possible but for the vision and eminence of some of our pioneers in various Ayurvedic systems of medicine, among whom I am glad to find the Rev. Malewana Gnanissara Thero, President of the Board of Indigenous Medicine. The place of Ayurveda in our national life has been securely established, and encouragement will be forthcoming from me personally in its further development (*Sir John Kothalawala, Prime Minster*)"[4].

"*It is with great pleasure I send a message to the Silver Jubilee Souvenir of the College and Hospital of Indigenous Medicine. The Hospital, College, and allied institutions have existed for 25 years. Amidst great difficulties the pioneer of these institutions supported it with a very small grant from the Government. During the period I was the chairman of the Board of Ayurveda I persuaded the government to take over these institutions to Government. This was done in 1941 and since then it has become an independent Department. From a meagre sum of Rs.50, 000, today it gets grants of over Rs. 1,000,000. There is still much to be done to resuscitate this science. I can visualize, as I did when I put it under Government, a great future for it in the medical services to the free people of Sri Lanka* (*S.W.R.D. Bandaranayake, Leader of the Opposition*)" [5].

The excerpts reveal that both the government and opposition supported the promotion of TM, and indeed TM had been a popular theme on election platforms due to the continuous nationalist campaign during that time. Bandaranayake, in particular, had been an ardent promoter of native culture in general, and TM in particular, from the very beginning of the revival activities during British rule in the late 1920s. Kothalawala too had taken a keen interest in developing TM. The Rev. Malawana Ghnanissara stated that Kothalawala was the first one to make an official visit to the College as Prime Minister and met a crowd assembled to petition him to improve the institutes in 1954. On his return, Kothalawala requested the then Minister of Health, E. A. Nugawela, to submit a report about the College and Hospital before his cabinet [6].

At the event, politicians from both the government and opposition made speeches encouraging the initiatives taken by Lenora and the Board to promote TM. For instance, Sir John Kothalawala said that;

"*Physicians in any system of medicine should not engage in professions like medicine for economic gains but for the welfare of patients. If so, medicine would develop within a very short period of time. In the past, the so-called western physicians treated TM practitioners like dogs. At present, there is no such thing, but it happened during colonial rule. All countries welcome new things. There is no limit to science. Therefore, both western and TM should develop in-hand-in hand together for the benefit of the future generation*" [7].

Presenting the progress report of the College for the past twenty-five years, Lenora stated that while it had developed certain areas, in some aspects it had failed to meet the goals set by its forefathers. He went on to state that;

"*One need not go further than the College of Indigenous Medicine to understand the narrow ideas that*

> *have prevailed. The institution, of which the Silver Jubilee is celebrated today, has been in existence for the last 25 years. But, as yet, there are no facilities for X-ray, pathological work, post-mortem examinations, etc., in spite of a Post-mortem room being the place where the over-jubilant medical man comes down to earth on many an occasion"*[8].

Furthermore, he complained that although a proposal had been presented to the government two years earlier, elaborating on how it should act in developing TM, nothing had been done except to change the duration of training at the College from four years to five years. At the same time, he stated that even though the Minister of Health was willing to implement some of the recommendations that he proposed, there were some TM based physicians who were opposed to such attempts of the government, fearing that they would lose the vested interests of their practice. At the end, he noted that the College had been able to produce 429 graduates despite all the problems that it had faced (it had in addition provided training to twenty eight more students under the five-year plan). By 1954, there were thirty-two academic staff members at the College, encompassing all three sections of TM, Ayurveda, Siddha, and Unani, and fourteen informally trained TM Specialists, three Special lecturers, two General Scientists, and five Western Medicine Lecturers [9].

The Jubilee-related speeches and texts show that TM was intertwined with national politics. The Jubilee celebration seems likes a large political event bringing politicians from the government and the opposition, along with college authorities, bureaucrats, practitioners, foreign dignitaries, and the public. Politicians from both sides anticipated that support to promote TM would be politically beneficial. Arguably, Lenora was keen to turn the Jubilee into a major political event to gain support for the reforms proposed by him under the Lenora system. The invitation of politicians from the government and the opposition, as well as other dignitaries can be considered a strategy applied by Lenora to weaken opponents of the Lenora System.

The Jubilee celebration did not see any of the positive results expected by Lenora and organizers of the event. The groups who had protested quietly against the Lenora system came forward and accused him and his supporters of trying to destroy TM. The opposing groups consisted mainly of informally trained TM practitioners.

Their main concern was that if the two medical systems should be integrated, it would pave the way for many disqualified TM practitioners to abuse the medical profession by prescribing more Western medicine for personal gain[10]. Stressing that their paramount duty was to safeguard TM, they criticised the intent of the proposed TM reform agenda to wipe out Ceylon's original form of TM [11]. The opposition was strong enough to hinder the implementation of the Lenora System. Meanwhile, the Kothalawala government introduced another Sessional paper to parliament in 1955 [12].

The opposing groups became even more vociferous when the Minister of Health, E. A. Nugawela, presented the *Ayurveda Dhawala Pathrikawa*, (Ayurveda White paper, or the Proposals for Revival and Development of Indigenous Medicine in Ceylon) before Parliament for its approval in 1955[13]. The minister presented the paper in keeping with the promise made by Kothalawala when he visited the College in 1952. The Ayurveda white paper was a synthesis of all previous Sessional papers and committee reports, and included long-term recommendations on how to overcome the problems faced by TM with the rapid spread of Western medicine in Ceylon. However, the contents of the white paper were based on the recommendations proposed by the Lenora System in 1952. Critiques of the paper were particularly passionate partly because it was printed only in English; they thought that the government was purposely making it difficult for informally trained practitioners, who represented the

bulk of the opposition, from accessing this information. Responding to this concern, the government translated it into Sinhala, and had it published in the Sinhala daily *Lankadeepa* newspaper's *Vedaparapuara* column on the 04th of June 1955[14].

The Ayurveda white paper proposed the following:

(I). A research division should be established at the College to conduct research on TM. In order to formalize the required tasks for such a unit, a committee should be appointed comprising the principal of the College, the chairman of the Board of Indigenous Medicine, the examiner of Materia Medica of the College, and the examiner of the Materia Medica of the Ceylon Medical College.

(II). Permission should be given to graduates of the College to use western medicine, and it ought to be authorised by the director of health services. In order to implement this practice, the necessary training should be given to students when they are at the College.

(III). A Candidate should have one of the following qualifications in order to enter the College:

a. either the Sinhala or Tamil Senior High School Examination Certificate with two distinctions in the following subjects: either the Sinhala or Tamil languages; Sinhala or Tamil literature, and the Sanskrit language. If a candidate could not obtain these distinctions in the Senior High School Examination, they could be obtained in the Senior Secondary Examination;

b. either the Intermediate Examination Certification of the Colombo or the Jaffna Oriental Society; and

c. either the Vidyodaya or the Vidyaylankara Pirivenas Examination Certificate.

(IV).

a. All Ayurveda, Siddha, and Unani traditions of medicine should be taught at the College;

b. A committee should be appointed to advice and design step-by-step-the proposed Siddhayurveda College in Jaffna;

c. The quality of teaching in Unani section should be improved at the College of Indigenous Medicine in Colombo.

(V). Buildings of the College should be renovated and material and instruments should be provided where they are required.

(VI). Buildings of the Hospital should be renovated and material and instruments should be provided where they required.

(VII).

a. A qualified Indian pharmacologist should be employed under the Colombo Plan scholarship to supervise Ayurveda pharmaceutical factory in the country.

b. A graduate of the College of Indigenous Medicine should be awarded a scholarship in pharmacological chemistry to visit India for training in the preparation of *Ayurvedic* pharmaceuticals;

(VIII). The Board of Indigenous Medicine should be abolished and, be replaced with the Department of Indigenous Medicine under the Ministry of Health[15].

The recommendations proposed by white paper did not indicate any significant departure from similar attempts made to promote TM in the past. As mentioned earlier, it was a combined proposal developed by the government in line with previous committee reports and the Lenora System. The motive behind the government move was not

known. Arguably, the UNP wished to gain more popular support from the ongoing Sinhala Buddhist nationalist activities to persuade the people that the UNP was not an anti-nationalist and anti-Buddhist party, although it had been labeled as an anti-patriotic party and a puppet of the western rulers.

The anti-reformist and anti-modernist TM practitioners (informally trained TM practitioners) took advantage of the re-emerging nationalist movement's popularity and objected to any government reform measures. Informally trained physicians mobilized in many parts of the country to defeat the Ayurveda white paper, and received help from prominent political leaders such as Bandaranayake. They used anonymous popular newspaper columns, *Vedana*, in the daily *Lankadeepa* paper, to popularize their views [16]. They also dedicated another column, known as the *Vedagedara*, in the daily *Lankadeepa*, to record their opposition against the government decision to modernise TM in Ceylon. In addition, they organised an ad-hoc interest group, *Veda hatana* (struggle of traditional practitioners). The newly formed group was different from the previous group[17]. In an interview with Registrar of the Sri Lanka Ayurveda Medical Council, he mentioned that the newly formed group was heterogynous when compared to the previous group. The new group comprised members of the Ceylon Ayurveda Congress, some graduates of the College of Indigenous Medicine and Gampaha Siddhayurveda College. In addition, they unionised their All Ceylon Ayurveda Congress whose anti-modernization campaign worked against the government decision to bring about the recommended changes[18].

The *Lankadeepa* published news items related to the contents of the white paper and the protest campaign on a daily basis from its inception until the *white paper was* defeated in 1956. The then editor of the *Lankadeepa*, D. D. Dhanapala, extended strong support to the anti-white paper campaign, and backed prominent leaders in the field of TM. They included, Ariyadasa Kumarasinghe, R. Buddhadasa, Gunasekare Owitigala, G. H. D. Kumaradasa, K. D. P. Piyasena, Pandit G. P. Wickramarachchi, and T. William Fernando. They opined that TM would lose its traditional identity once it was integrated into modern medicine, as was allegedly the case of the Ashthanga Ayurveda College in Calcutta. In addition, the well-known Indian Ayurvedist, Pandit Shiv Sharma, who was in Ceylon at that time, also joined the protest movement to safeguard the original form of TM medicine [19].

Some members printed personal magazines to express their opposition to the white paper. For instance, Millawe Ariyapala, then a well-known informally trained TM practitioner, published a monthly magazine, namely, the *Vadakaha*, and recorded his and some of his colleagues' objection to the reforms proposed by the white paper [20]. In the October edition, 1955, he published amendments proposed by the All Ceylon Ayurveda Congress against the government's attempt to modernise TM.

The Ceylon Ayurveda Congress opined that Sinhalese-English or mixed medicine practice should be eradicated and measures taken to expel corrupt TM practitioners from the country. To minimise professional competition between western and TM practitioners, the two traditions of medicine should be treated equally. Traditional recipes should be given priority to promote prevention of illness as a long-term measure. The Ceylon Ayurveda Congress proposed that only western trained doctors should be allowed to recommend allopathic medicine to the public. It was also of the view that dispensaries, both western and TM, should be set up all over the country to provide a better drug-delivery service to the people. A ban was proposed on the import of drugs, and the public were encouraged to use locally produced drugs to save foreign exchange. Finally, the Ayurveda Congress came up with a proposal that poorly trained doctors of either tradition of medicine should be banned from serving in the government health service [21].

The objection to the training provided by the College of Indigenous Medicine to students suggests that informally trained TM practitioners held negative views. The last recommendation points to their judgment that in order to maintain harmony between the two traditions of medicine and protect the public, quacks should be discouraged from working in government hospitals.

At the same time, some groups who labeled themselves 'progressives' supported the government's Ayurveda white paper. They included the Indigenous Medical Association, the Jaffna Siddha Medical Association, Southern Sri Lanka Indigenous Medical Association, and the Sabaragamuwa Indigenous Medical Association. Students, both former and contemporary, of the College of Indigenous Medicine, who supported the proposals in the white paper, and organized protest campaigns attacking the anti-reform groups, joined them. Furthermore, the Indigenous Medical Association, a union of former graduates of the College, unanimously took a decision to terminate their relationship with the All Ceylon Ayurveda Congress, an informally trained TM physicians' professional union, for switching their allegiance from supporting Lenora to opposing the white paper. Despite the support it received, however, the government decided to withdraw the proposal in 1956[22].

The Lenora System of Traditional Medicine Reforms

While the government abandoned the reforms put forward by the white paper, the members of the Board of Indigenous Medicine did not show any sign of giving up on the reforms proposed by the Lenora System. To gain support, the chairman of the Board, the Rev. Malewana Gnanissara, printed a small booklet on the important issues highlighted by Lenora in 1954. He titled it 'Towards Progress of Ayurveda', and into it he incorporated some correspondence between Lenora and prominent figures on a variety of subjects in the field of TM in India and Ceylon [23]. The pamphlet intended to clarify existing misconceptions that the people, professionals, students, and some politicians had about the integration of Western medicine with TM in Ceylon.

Lenora believed that people suffered unnecessarily from their reliance on TM drugs, and if Western medicine could be prescribed at the Indigenous Medicine Hospital this suffering would be alleviated. He stated that 'personally, I feel that it is a grave offence to allow people to die whilst there are drugs that can cure them and alleviate their suffering' [24]. He opined that western-trained practitioners denied people's right to use western drugs at the Indigenous Medicine Hospital. He criticised them, asking;

> "*What right has a section of doctors to deny these to our countrymen, and ask for a monopoly of these drugs? If they have the national feeling, they should be the leaders in the field and teach the lesser-informed brother of all recent advances irrespective of other petty considerations. Yet these are the very people who have taken the 'Oath of Hippocrates' to alleviate human suffering"*[25].

Lenora further argued that it was with the best of intentions that he had proposed the proper usage of western drugs in his scheme, but a segment of western doctors voted against it. He mentioned that 'those who are against give no reasons except that Ayurveda should be kept within the narrow confines of what was taught 3000 years ago. But these very people have no objection to the use of modern drugs by Apothecaries who get a two years' training or to their use by Estate Dispensers [26].

Lenora explained that the Ceylon Medical Council regulations discouraged TM practitioners from using modern drugs in their practice. He described the deeply rooted prejudice held by western medicine practitioners towards their counterparts. He stated that;

> "*The Medical Council regulations threaten qualified doctors that their names will be erased from the Register if they associated with Ayurvedic physicians or teach the Ayurvedic students. The government has recognised these practitioners, and the Institution belongs to the government. To say that the government is sponsoring quackery is a highhanded action of the Ceylon Medical Council*"[27].

Lenora also identified western physicians who thought that allowing TM practitioners to prescribe western drugs was a reasonable practice to save patients at the Indigenous Medicine Hospital. He listed O. E. R. Abeyrathne, P. R. Anthonis, S. W. Bibile, Professor C. C. De Silva, Hilary Gunawardene, G. R. Handy, Professor, J. H. F. Jaysooriya, Kumara Rathnam, L. S. C. Mendis, Sir Nicholas Attygalle, A. Nimalsuriya, G. S. Sinnathamby, R. P. Wijerathne, and J. R.Wilson, who were sympathetic to his proposal to use Western medicine at the Hospital[28].

Lenora was surprised to find out that some informally trained TM physicians fiercely opposed allowing TM practitioners at the Hospital to prescribe Western medicine. He said the members of the Ceylon Ayurveda Congress contradicted themselves when they supported the teaching of western sciences to students of the College of Indigenous Medicine but do not want them to prescribe western drugs. He stated that;

> *"The official of the Congress have raised objections and some even publicly expressed that even life should be sacrificed to preserve Ayurveda in its pure form. Yet, in a memorandum to the Hon. the Minister of Health, they have demanded that all aspects of modern medicine, including the whole pharmacopoeia, be taught at the College, but that the use of modern drugs be prohibited. In the very next page they say, 'No doctor be allowed within the premises as he is bound to ruin Ayurveda. If this were put into effect they do not point out who is to teach Anatomy, Physiology, etc., on which they insist. It is a pity that not a single office bearer of the Congress has been trained in a Hospital, or has ever worked in a Hospital. Therefore, they are neither conversant with the difficulties of Hospital Practice nor how to overcome these difficulties"* [29].

At the end of the pamphlet, the Rev. Gnanissara is quoted as saying that amidst fierce opposition by various groups against reforms, Lenora was keen to maintain correspondence with Indian universities and colleges to determine how they conducted their TM teaching practices and incorporated modern sciences and medicine. The pamphlet included a large number of such communications. For example, Lenora sent a letter on the 22nd of February 1956 to the principal of the College of Indigenous Medicine in Madras inquiring whether the College recruited western allopathic staff and taught Western medicine. In reply, the principal stated that 'I am to inform you that the entire Materia Medica of Modern Medicine is taught to the third year students of the College of Indigenous Medicine, and also Pharmacology and Therapeutics. They are also given lessons in practical dispensing' [30].

The Rev. Gnanissara included in the booklet a letter from Pundit G. P. Wickramarachchi, founder of the Siddhayurveda College of Gampaha. At the beginning, Wickramarchchi was supportive of the Lenora System, but in 1955 led the anti-white paper campaign. The letter sent by Wickramarchich to Lenora on the 21st of September 1952 stated that 'I am pleased to contribute anything for the genuine effort that you have undertaken to develop TM in the country, and wish you success in implementing your plan [31].

The contents of the translated excerpts of Wickramarachchi's letter indicate that he contradicted his opposition to the white paper. By exposing Wickramarachchi's ardent support for reform, Lenora likely sought to undermine his current opposition to his programme. It is unclear why Wikramarachchi changed his position from being a supporter of the Lenora system to an opponent. It is likely that the growing Sinhala Buddhist nationalist campaign led by Bandaranayake influenced Wickramarachchi to change his mind. As will be revealed later in this letter, his close association with nationalist groups paved the way for him to become a Senate member in 1957, to receive government financial support to maintain the college, and to secure employment opportunities for his College graduates.

Finally, Lenora quoted the following excerpt from the speech, '*The modern system of medicines,*' made by the first prime minister of India, Shri Jawaharlal Nehru, to the Health Ministers' Conference, New Delhi on the 31st August 1950, in support of his endeavour to modernise TM in Ceylon;

> "*What then should our approach be? Obviously, our approach should be one of trying to profit by past experience and integrating it with the least in other systems. One approach, I would for want of a better word, call, the 'Scientific Approach', the approach of a knowing mind, and experimenting mind, which is preferred accept any thing that factually or theoretically justifies itself and which goes ahead on the basis of it. When something else takes its place as an improvement, a scientific mind accepts that in theory at least; what is called modern medicine is based on this Scientific Approach"* [32].

Lenora and the pro-reformists members of the Board of Indigenous Medicine included excerpts from correspondence to convince the public, government, and professionals in the field to support their TM modernization programme. The use of the quotation from Nehru can be considered a strategy employed by the members of the Board to justify their struggle in the ongoing debate on how to reform TM. In addition, the Board would have anticipated that Nehru's statement on the scientific approach could weaken the resistance of groups opposed to the reform of TM.

The pamphlet also highlighted the major difference between Lenora and his supporters of reform and members of the opposition. The former were modernists, who drew on the modern scientific approach to justify their struggle to reform TM in Ceylon. The latter were Sinhala Buddhist nationalists who justified their struggle by drawing on the historical past. However, Lenora and his supporters did not have much respect for the opposition, referring to them as mules: 'the physician who does not learn is compared to a mule, who carries a load of sandalwood realising only the weight of the load, but not the value of what it carries'[33]. Arguably, this shows the negative perception held by the modernists towards TM practitioners, who rejected the integration of some elements of the two systems of medicine to modernise TM.

Meanwhile, the Rev. Gnanissara and Lenora realised that neither the Lenora system, nor the white paper would be implemented anytime soon. As a result, the former resigned from the Chairmanship of the Board in October 1956. Subsequently, the government appointed Mr. S. Amarasinghe (Chairman of the Board). The other Board members were: P. M. P. Abeysinghe, C. Amarasinghe, A. M. M. Ameez, M. C. Chandrasena, S. D. S. Kelanithilake Gunawardane, A. P. Samather, G. K. D. I. Senarath, Muhandiram, Mr. C. S. Wettasinghe, and A. W. Wijerathne Kavichinthamani [34].

Disappointed, Lenora resigned from his post as the principal of the College on the 25th of January 1957[35]. Immediately after, a group of students began boycotting classes and demanding that the

government reinstate the principal. Instead, it chose the deputy principal, J. E.P. Wickramasinghe to be the acting principal. The new acting principal issued three ultimatums to the students, and struck off the names of those who failed to return to the College from the register of students[36]. Later, Lenora arranged a special scholarship programme in consultation with the Medical Fund for the affected students in the strike of 1957, to enable them to pursue higher education at Indian universities[37].

Conclusion

In conclusion, it could be argued that a majority of informally trained TM practitioners opposed these reforms, not at least as they feared a decline in their professional status. Although, they did not question the necessity of reforming TM, they were sceptical about the policies being deployed by successive government to restructure education, training, and practice of TM. They demanded that *Desheeya Chikitsa* be given greater consideration by the authorities, so that it was formally recognised alongside Ayurveda, Siddha, and Unani. They highlighted the need for *Desheeya Chikitsa* to grow in par with other medical traditions. However, their demands were largely disregarded during the colonial period, in the situation where government and college authorities were unwilling to accommodate informally trained TM medical practitioners as teaching staff and introduce academic courses on *Desheeya Chikitsa*. Instead, the informally trained practitioners were allowed to operate outside the mainstream, which enabled them to provide practical training to students within their practices.

The post-independence situation was equally mixed. Reforms in TM education were rather ad-hoc, with the new national governments initially continuing changes according to a blueprint set out by the proceeding colonial authorities. The focus of reforms was, therefore, what can be referred to as the 'biomedicalization' of TM; an approach that continued to stoke tensions among politicians, administrators, and TM practitioners. As it is seen, there were two competing groups, one was anti-reform and the other was pro-reform. Their disagreements and debates made, TM a highly contested political issue, with opposing views being taken up and advocated by political personalities and parties.

S.W.R.D Bandaranayake's political statements and actions best exemplify the politically charged nature of discussions about reforms in TM. He initially advocated the promotion of *Desheeya Chikitsa*, which allowed him to mobilise the support of informally trained TM practitioners. However, after coming to power through a closely contested election he changed his priorities -- he decided that it was better to rely on Indian expertise to revise frameworks for education and practice in TM. Many interpreted this as a betrayal of Sinhala nationalism.

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Ajita Agada for Poisoning Conditions and Interpret its Mode of Actions

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Abstract

Poisonings have been identified as critical conditions in Ayurveda since centuries. *Agada tantra* is the specialized clinical branch where the therapeutic roots for poison management lie. *Agada* are given a significance as anti-poisonous formulations in various Ayurveda treatises. *Ajita agada* is the main concern in this study which the references were found on Susruta Samhita *Kalpasthana*, Aṣhṭanga Samgraha *Uttarasthana* and Bhaisajjaratnavali. This herbo-mineral anti-poisonous formulation is consisted of 17 ingredients and bee honey as its dipping material. *Ajita agada* is prescribed mainly for snake bites (*Sarpa visha*) and also for all the other kinds of animate (*Jangama*) and inanimate (*Sthavara*) poisons. Still, any organized management procedure in critical care for poisonings from Ayurveda perspective hasn't observed included in national health care system of Sri Lanka. This study is aimed at fulfilling this lacuna by means of finding a strategy for critical care of poisonings through *Ajita agada*. Upon Ayurveda pharmacodynamics are concerned, it's observed that *Kaṭu* (76.47%), *Tikta* (35.29%) and *Kashaya rasa* (29.41%) are prominent *Rasa*, *Laghu* (94.11%), *Tikshṇa* (58.82%) and *Ruksha guna* (35.29%) are prominent *Guna*, *Ushṇa* (82.35%) and *Katu* (23.52%) as the prominent *Virya* and *Vipaka*. By virtue of pharmacodynamics, *Ajita agada* shows a similarity with poison itself. This is very remarkable in collective understanding the therapeutic action of *Ajita agada* in management of poisonings. Further, chemical and clinical studies should be conducted with this regard.

Keywords: *Ajita agada,* Anti-toxicity, *Visha, Visha upakrama.*

Introduction

Ayurveda is an organized medical system with a ruler strong foundation of eight clinical branches, namely *Ashṭanga* Ayurveda[1]. Among these eight clinical branches, *Agada tantra* broadly elaborates Ayurvedic perspective of toxicology. In present circumstances, toxicology is practiced as a medical sub speciality to diagnose, manage, treat and prevent poisoning conditions. Similarly, all these concepts practiced in present scenario are vividly denoted in *Agada tantra*. References are found for sources, modes of administration, classifications, management, treatment procedures and prognosis for various kinds of poisonings from ancient Ayurveda authentic texts. This well depicts the fact that ancient *Acharyas* had identified the critical need of management of poisoning conditions.

As Ayurveda is a medical system based on natural substances, a line of treatment from medicines comprised of natural herbals and minerals for the management of poisonings are compiled on ancient Ayurveda treatises. These anti-poisonous formulations are given the name, "*Agada*" which are naturally prepared herbal or herbo-mineral drug combinations. In references, preparatory methods, addressable poisoning conditions, other clinical indications, mode of administration, dosage form and vehicles are provided with these formulations.

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In present scenario, poisonings have become a critical condition that is to be managed immediately. Mortality rates attributed to unintentional poisonings in males and females in the year 2019 are 0.7 and 0.2 respectively in Sri Lanka[2]. These rates are identified remaining relatively high in low economy countries[2]. Snakebites are one of the common unintentional poisonings and the annual snakebite incidence in Sri Lanka is about 400 per 100,000 people corresponding to 80,000 snakebites in 20 million population[3].

Agada tantra describes these poisoning conditions as manifestations of features of poisonings (*Visha lakshana*) as the result of intaking poisonous substances (*Viha dravya*) in excessive quantities (*Atimatra*), ignorant ingesting/inhaling (*Mityayoga*), impurified conditions (*Ashodhana*) and excessive duration of exposure (*Adhika kala*) [4].

Ajita agada is such an anti-poisonous preparation in which the references were found on Susruta Samhita *Kalpasthana*, Aṣṭangasamgraha *Uttarasthana* and Bhaisajjaratnavali. This herbo-mineral anti-poisonous preparation is consisted of 17 ingredients and bee honey as its dipping material and the storage medium. *Ajita agada* is prescribed for all kinds of animate (*Jangama*) and inanimate (*Sthavara*) poisons, providing a justification for its literal meaning – conquering, being invisible[5] besides mainly indicated for snake bites.

Modern toxicology emphasizes the utility of antidotes. This concept was being molded along with the progression of understanding the mechanism of poisons[6]. Antidotes are understood as agents which nullify the toxic effects[7]. Antidote action is mediated in major two ways, either preventing the absorption of the toxin by binding with it or inhibiting the metabolism of toxins into more toxic metabolites inside the body[7].

Still, any organized management procedure in critical care for poisonings from Ayurveda perspective hasn't observed included in natural health care system of Sri Lanka. This study aimed at reviewing *Ajita agada* which is indicated for all kinds of poisons and consisted of easily available ingredients and a simple preparatory method to find out a strategy for addressing the critical care of poisonings. And also this study was aimed to review on *Ajita agada* for poisoning conditions and analyze the pharmacodynamic properties and interpret the mode of its action and discussed on preparation of *Ajita agada* as per Ayurveda authentic texts.

Materials and Methods

Data required for the review was collected from relevant published review articles and available Ayurveda authentic texts and associated books. Credible web search engines such as ResearchGate, Google Scholar and PubMed were utilized during the study.

Available Ayurveda authentic texts: Susruta Samhita, Charaka Samhita, Aṣṭanga Saṃgraha, Bhaisajjaratna-vali were used. And other relevant books used: The Ayurvedic Pharmacopoeia of India-Part 1 (Volume i-iv), Dravyaguna Vijnana (Materia medica-Vegetable, drugs) by Prof. G. Pandey (Volume 1-3).

Table 1 shows the authenticated ingredients of *Ajita agada*. Among all 17 ingredients, 12 ingredients are of herbal origin and the rest is of mineral origin. Five varieties of salts (*Lavana*) are the mineral origin ingredients of the preparation. Bee's honey (*Madhu*) is the storage medium used in the preparation process.

Method of Preparation

Authenticated raw materials were spread out in a thin layer in trays and the foreign matters were detected by inspection with the naked eye and by the use of a magnifying lens (6x). Washing and drying of herbal raw materials were undertaken; washed in tap water and shade dried on trays (to avoid destroy of aromatic compounds). Relevant purification methods were followed for *Hingu* and *Lavana varga* (salt varieties). *Hingu* was emulsified in sufficient quantity of water and each salt variety was filtered and evaporated. Dried raw materials were ground separately in a grinding machine and passed through the No 180 sieve and a fine powder was obtained (Figure 1).

Table 1: Ingredients of *Ajita agada*

Ingredient	Scientific name	Family	Used part	Quantity
Viḍanga	*E. ribes*	MYRSINACEAE	Fruit	50 grams
Paṭa	*C. pareira*	MENISPERMACEAE	Roots	50 grams
Haritaki	*Terminalia chebula* Retz.	COMBRETACEAE	Fruit	50 grams
Amalaki	*Embellica officinalis* Gaertn	EUPHORBIACEAE	Fruit	50 grams
Vibhitaki	*Terminalia belerica* Roxb.	COMBRETACEAE	Fruit	50 grams
Shunṭi	*Zingiber offficinale* Roscoe	ZINGIBERACEAE	Rhizome	50 grams
Maricha	*Piper nigrum* L.	PIPERACEAE	Fruit	50 grams
Pippali	*Piper longum* L.	PIPERACEAE	Fruit	50 grams
Hingu	*Ferula asafoetida* L.	UMBELLIFERAE	Resin	50 grams
Ajamoda	*Trachyspermum ammi* L.	UMBELLIFERAE	Seed	50 grams
Citraka	*Plumbago zeylanica* L.	PLUMBAGINACEAE	Roots	50 grams
Tagara	*Valeriana walichi* DC	VALERIANACEAE	Roots	50 grams
Saindhava lavana	-	-	-	50 grams
Sauvarcal lavana	-	-	-	50 grams
Samudra lavana	-	-	-	50 grams
Vid lavana	-	-	-	50 grams
Romaka lavana	-	-	-	50 grams
Bee's honey	Apis cerana Fabricius	APIDAE	-	As necessary

Figure 1: Separately obtained powders from all ingredients

Figure 2: Final homogenous powder mixture

Figure 3: The final homogenous powder mixture with bee's honey added in an adequate amount

Figure 4: Preparation ready for storage inside the cow's horn containing bee's honey

The final homogenous powder was obtained by mixing all the fine powders (Figure 2) and dipped in an adequate amount of bee's honey (Figure 3).

Finally, the paste (*Kalka*) form obtained after the process of dipping was stored in a cow's horn with a lid made of the same material for two weeks (Figure 4).

Results

Three sources were found consisting references for *Ajita agada*: *Sloka* 63(ii)-65(i) of Chapter 5 of Susrutasamhita *Kalpasthana, Sloka* 101 of chapter 40 of, Aṣṭanga Samgraha *Uttarasthana* and *Sloka* 41-42 of chapter 72 of Bhaisajjaratnavali.

All the references were comprised of the ingredients, preparation method, and indications of the preparation. Being a herbo-mineral preparation according to the given recipes, *Ajita agada* was composed of twelve herbal materials and five varieties of salts (*Lavana*) were of mineral origin. Bee's honey which is of animal origin was the grinding material. A specific storage method by using a cow's horn was mentioned in the references from Susrutasamhita [9] and Bhaisajjaratnavali[10].

The pharmacodynamic (*Rasadi panchaka*) analysis which was done during the study is given in table 2. Taste (*Rasa*), attributes (*Guna*), potency (*Virya*), post-digestive effect (*Vipaka*) and effects on three humors (*Dosha karma*) were considered under the pharmacodynamic analysis.

When *Ayurveda* pharmacodynamics are concerned, it's observed that pungent taste (*Kaṭu rasa*) which was 76.47%, bitter taste (*Tikta rasa*) which was 35.29% and astringent taste (*Kasaya rasa*) which was 29.41% were the prominent *Rasa* of the preparation. Lightness *(Laghu*) which was 94.11%, Sharpness (*Tikshṇa*) which was 58.82% *and* Roughness (*Ruksha*) which was 35.29% were the prominent *Guna.* Among *Virya,* hot potency (*Ushṇa virya*) which was 82.35% and among *Vipaka.* Pungent (*Katu vipaka*) which was 23.52% were observed as the prominent. Pacification of both *Kapha* and *Vata* (*Kapha-vata shamaka*) which was 55.56% was the prominent *Dosha karma* of the ingredients of *Ajita agada.*

Table 3 depicts the reported modern pharmacological actions of herbal ingredients in *Ajita agada*. Antioxidant, anti-inflammatory, analgesic, cardioprotective and hepatoprotective were the prominently identified pharmacological actions.

Anti-inflammatory action was found in bee's honey[37] and *Saindhava lavana*[38] Further immunomodulatory actions were found in *Plumbago zeylanica*[39], *Trikatu*[40], and *Triphala*[41] in-vivo experimental studies.

As far as the *Ajita agada* is concerned, the special reference from Susruta Samhita *Kalpasthana* chapter five which is *Sarpadastavisha chikitsa kalpa* by name and dedicated for treatments for snake bites, immunomodulatory action of the ingredients of *Ajita agada* is very remarkable.

Also, it's observed anti-venoms are utilized as a special treatment to manage this critical condition immediately. Anti-venoms act by inducing immunity by binding with the venom for neutralization[42]. Versions of anti-venoms are available for spider bites, snake bites, fish stings and scorpion stings[43].

Though the antivenoms are promised with lowering the mortality from snake bites and highly answerable for the critical management of such conditions, some adverse effects are also been identified. Blood-clotting problems, muscle injury, hypotension leading to shock, kidney damage, neurology problems, severe allergic conditions, swelling and serum sickness are such adverse effects[44]. Also, production of anti-venom is highly cost and lacuna of suitable animal models may be occurred[6].

Table 2: Pharmacodynamic properties of the ingredients of *Ajita agada* according to Ayurveda[11.12]

Ingredient	*Rasa*	*Guna*	*Virya*	*Vipaka*	*Dosha karma*
Vidanga	*Kaṭu* *Kashaya*	*Laghu, Rukṣha,* *Tikṣṇa, Sara*	*Ushna*	*Katu*	*Kapha-vata shamaka*
Paṭha	*Tikta*	*Laghu* *Tikṣṇa*	*Ushna*	*Katu*	*Kapha-vata shamaka*
Haritaki	*Madhura* *Amla, Kaṭu* *Tikta, Kaṣhaya*	*Ruksha* *Laghu* *Sara*	*Ushna*	*Madhura*	*Tridoshaghna* *Vatashamaka*
Vibhitaki	*Kashaya*	*Laghu* *Ruksha*	*Ushna*	*Madhura*	*Tridoshaghna* *Kaphaghna*
Amalaki	*Madhura* *Amla, Kaṭu* *Tikta, Kashaya*	*Ruksha* *Sara* *Guru*	*Sita*	*Madhura*	*Tridoshaghna* *Pittahamaka*
Shunti	*Katu*	*Laghu, Snigdha*	*Ushna*	*Madhura*	*Kapha-vata shamaka*
Marica	*Kaṭu*	*Laghu, Ruksha* *Tikshṇa*	*Ushna*	*Katu*	*Kapha-vata shamaka*
Pippali	*Kaṭu* *Madhura*	*Laghu, Snigdha*	*Anushna*	*Madhura*	*Kapha-vata shamaka*
Ajamoda	*Katu* *Tikta*	*Laghu, Ruksha* *Tikshṇa*	*Ushna*	*Kaṭu*	*Kapha-vata shamaka,* *Pittavardhaka*
Hingu	*Katu*	*Laghu* *Rukhṣa* *Tikshṇa*	*Ushna*	*Kaṭu*	*Kapha-vata shamaka*
Citraka	*Kaṭu*	*Laghu, Ruksha* *Tikshṇa*	*Ushna*	*Kaṭu*	*Kapha-vata shamaka*
Tagara	*Tikta, Katu,* *Kaṣhaya*	*Laghu* *Snigdha*	*Ushna*	*Kaṭu*	*Kapha-vata shamaka*
Madhu	*Madhura Kaṣāya*	*Laghu, Ruksha* *Yogavahi*	*Sita*	*Kaṭu*	*Kaphaghna*
Saindhava lavana	*Lavana* *Madhura*	*Laghu, Snigdha* *Tikshṇa*	*Sita*		*Tridoshaghna*
Sauvarchal lavana	*Lavana* *Kaṭu*	*Laghu, Snigdha* *Sukshma*	*Ushna*	*Katu*	*Vatahamaka*
Samudra lavana	*Lavana* *Madhura* *Tikta, Kaṭu*	*Guru, Snigdha* *Tikshna*	*Ushna*	*Madhura*	*Vatashamaka*
Vid lavana	*Lavana*	*Tiḳshna, Vyavai* *Laghu*	*Ushna*	*Madhura*	*Vatashamaka*
Romaka lavana	*Katu, Tikta* *Katu*	*Laghu, Thikshna* *Sukshma, Vyavai*	*Ushna*	*Katu*	*Kapha-vata shamaka*

Table 3: Reported modern pharmacological actions of the ingredients of *Ajita agada*

Latin name	Biological activity	References
Embelia ribes Burm.f.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	13, 14
Cissampelos pareira L.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	15,16
Terminalia chebula Retz.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	17,18
Embellica officinalis Gaertn	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	19,20
Terminalia belerica Roxb.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	21,22
Zingiber offficinale Roscoe	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	23,24
Piper nigrum L.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	25,26
Piper longum L.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	27,28
Ferula asafoetida L.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	29,30
Trachyspermum ammi L.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	31,32
Plumbago zeylanica L.	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	33,34
Valeriana walichi DC	Antioxidant, Anti-inflammatory, Cardioprotective, Hepatoprotective	35,36

Discussion

Ajita agada is a very simplified herbo-mineral preparation according to its references. Ingredients, preparatory method and indications were mentioned same in all three references whereas the storage method was only found in Susruta Samhita and Bhaisajjaratnavali.

Storing of the final *Kalka* inside a cow's horn with an excessive amount of bee's honey, has been mentioned as the preparatory method. A defined mode of administration, dosage form and an *Anupana* (Vehicle) for the preparation were not available in referred texts. But Venkatro, 2015 mentions *Ajita agada* can be administered internally in the treatments and complications of *Visha*. Also, according to an online resource, *Ajita agada* 12-24 grams to be taken with 100 to 250 ml. of milk twice a day mentioned as a general treatment for all types of *Viṣha*. *Ajita agada* is capable of administering in general *Kalkamatra* (one *Karsha*) and the *Anupana* should be decided upon the condition by the physician.

When Ayurveda pharmacodynamics are concerned, it's observed that *Kaṭu* (76.47%), *Tikta* (35.29%) and *Kaṣhaya rasa* (29.41%) are prominent *Rasa*, *Laghu* (94.11%), *Tikshṇa* (58.82%) and *Ruksha guna* (35.29%) are prominent *Guna*, *Ushṇa* (82.35%) and *Katu* (23.52%) as the prominent *Virya* and *Vipaka*. Almost all the attributes of *Visha* are observed in pharmacodynamics of the ingredients in *Ajita agada*. It is contradicted with the antagonistic properties to *Visha* observed in a typical anti-poisonous drug. Belvadi, 2019 states the availability of certain *Kashta aushada* providing *Viṣhokta lakshana* and simultaneously pacify *Visha*.

Dilipkumar, 2015 mentions being equally potent to *Visha*, possessing *Vyavai guna* to act vigorously on *Vishapiḍita* patient and having the same affinity for the system on which poison has affected are some of criteria of Acharyas elaborated to put forth a *Dravya* as a *Prativisha* (antidote). As Ayurveda pharmacodynamic properties (*Rasadi panchaka*), pharmacological properties (*Karma*), therapeutic indications (*Prayoga*) and also, the reported modern pharmacological actions being tallied above mentioned criteria, *Ajita agada* can be understood as an anti-poisonous preparation with some characteristics of *Prativisha* (antidote).

Being indicated for *Sthavara* (Inanimate) and *Jangama* (Animate) *Visha* is a special remark of *Ajita agada*. Pharmacological properties on both Ayurveda and modern perspectives are contributed in the management of general signs and symptoms of *Sthavara* (inanimate) and *Jangama* (animate) Viṣa, for which the *Ajita agada* is indicated.

Also, the availability of varieties of antivenom for different kinds of animal poisoning conditions such as fish sting, bee sting etc. tallies with one of the authenticated indications of *Ajita agada* for all kinds of inanimate poisons (*Jangama visha*).

Complications and organ damage due to chronic toxicity according to modern toxicology, are answerable from *Ajita agada* due to the reported pharmacological findings observed in its ingredients. Antioxidant, anti-inflammatory, cardio-protective, neuro-protective, hepato-protective and analgesic are some of modern pharmacological actions found on the ingredients in overcoming complications and organ damages. Remarkably these pharmacodynamic properties are capable of overcoming the adverse effects resulted by artificial anti-venom antidotes which were mentioned beforehand.

Pharmacokinetics of the *Ajita agada* can be understood on basis of its pharmacodynamics. Finely powdered ingredients provide a good absorption as the particle size is reduced. Majority of *Guna* such as *Laghu*, *Tikshṇa* and *Ruksha* aided in penetrating into subtle levels of *Srotas* (body channels) and resulting a good distribution. Also, *Yogavahi guna* in bee's honey is contributed in good distribution. The prominent *Katu vipaka* results in good drug metabolism. *Lavana varga* in the preparation help in elimination of the poison as well as the drug.

As per antidote studies in modern toxicology, the action of *Ajita agada* can be conceptually understood as blocking the site of toxin by preventing the further spread of the toxin.

Conclusion

Considering the mode of actions, being similar with *Visha* property wise, the preparation is capable of providing an antagonist effect by blocking the rapid distribution and onset of *Visha*. Also, the actions of *Ajita agada* would be an approach to the management of complications and organ damage due to chronic toxicity on the basis of reported modern pharmacological actions of its ingredients.

Further, chemical studies should be scoped with this regard to understand the mechanism of action of this anti-poisonous formulation. The strategy of developing *Ajita agada* into a cost reductive and side effects minimal pharmaceutical dosage form for management of poisonings from Ayurvedic perspective would be a new milestone.

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The Phytochemistry and Antioxidant Activity of the *Nelumbo nucifera* (Lotus) Plant: A Review

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Abstract

Plant extracts are used in a range of products, including fairness creams, fragrances, body lotions, and other formulations. Herbal plants comprise alkaloids, glycosides, terpenes, steroids, flavonoids, tannins, and other secondary metabolites. Polyphenols have antioxidant properties, which have an assortment of health benefits. Medicinal properties can be found in almost all parts of the *Nelumbo nucifera* (Lotus) plant. Phytochemicals, as well as secondary metabolites like phenols, flavonoids, tannins, alkaloids, sterols, terpenoids, cardiac glycosides, coumarins, and quinones, are found in the lotus extracts. Methanol and Butanol extracts of *N. nucifera* are observed to have scavenging activities on free radicals and hydroxyl radicals, as well as the metal-binding ability and reducing power. Natural antioxidant potential has been observed in the leaf, stamens, and seed extracts of *N. nucifera*. The antioxidant activities of various organic and aqueous extracts of *N. nucifera* have been demonstrated in pharmacological studies. Antioxidant activity and total phenolic content may differ depending on geographical location and growth conditions of the plant. Further research into the fine structures of lotus active ingredients, as well as structure-activity relationship mechanisms, are required in the future to overcome the challenges of different extraction methods and in the studies of therapeutic efficacy of the *Nelumbo nucifera* plant.

Keywords: *Nelumbo nucifera*, Phytochemistry, Antioxidant property, DPPH, Lotus extract, free radicals

Introduction

Plant components and extracts have long been employed in the pharmaceutical, cosmetic, and food industries. There are minimal or no detrimental side effects when plants as well as plant extracts are utilized in applications. Fairness creams, fragrances, body lotions, and other formulations containing plant extracts are popular[1,2]. Alkaloids, glycosides, terpenes, steroids, flavonoids, tannins, and other secondary metabolites are found in herbal plants. Polyphenols have antioxidant properties, which have a variety of health benefits[3].

Since ancient times, many of these herbal products and spices have served as useful medicinal sources for humans[5]. Metabolism as well as illnesses produce significant amounts of free radicals and Reactive Oxygen Species (ROS) which may lead to oxidative stress. Antioxidants are important to restore the oxidant balance in the body in such circumstances. Natural antioxidants are preferred due to their minimal risks and side effects. Various in vitro and in vivo methods have been used to estimate the antioxidant properties of various parts of the lotus until now. However, no single metric is thought to be sufficient in determining total antioxidant capacity. This review aims to explore some important aspects of the antioxidant properties in different parts of the *Nelumbo nucifera* plant.

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Botanical description of the Nelumbo nucifera plant

Nelumbo nucifera belongs to the Nymphaeaceae family of plants. It belongs to the order Proteales and genus Nelumbo. Indian lotus, water lily, and Chinese lily are some of the names by which this plant is recognized in various geographical locations[4,5]. Lotus is a perennial aquatic plant with a fleshy yellow rhizome, an elongated creeping stem, nodal roots, green fruits, and large leaves.

Lotus flowers come in two varieties: white flowers and pink or reddish-pink blossoms (Figure 1).

Figure 1: The *Nelumbo nucifera* flower[6]

Flowers are sweet scented, hermaphrodites, and solitary. The aggregate fruit of this plant has a tough, brownish, longitudinally striated pericarp, and a single seed contained within the fruit. The seeds are released by bending the pod down to the water. The leaves of this plant float on the water's surface, while the roots are anchored in the pond or river bed. Petioles are long, rough and prickled. The roots float in aquatic systems due to the presence of numerous air pockets that run the length of the tuber. Australia, China, India, Sri Lanka, Iran, and Japan are all home to *Nelumbo nucifera*. Lotus has evolved to thrive in moist and humid conditions such as flood plains and slow-flowing rivers. Medicinal properties can be found in almost all parts of the plant. The flower, seed, rhizome, and leaves are used to treat conditions such as smallpox, diarrhoea, cough, fever, and cholera. Ayurveda also uses these plant elements to treat nausea, leprosy, and skin disorders. It is commonly used to diagnose conditions like tissue inflammation, cancer, and skin disorders. Alkaloids, steroids, flavonoids, glycosides, and polyphenols are among the pharmacologically active phytoconstituents credited with these abilities[3–5].

Phytochemistry and constituents of Nelumbo nucifera.

Primary metabolites like carbohydrates, proteins, and lipids are found in *Nelumbo nucifera* extracts, as well as secondary metabolites like phenols, flavonoids, tannins, alkaloids, sterols, terpenoids, cardiac glycosides, coumarins, and quinones (Figure 2)[4,5].

Nuciferine, pronuciferine, lotusine, rutin, hyperin, and demethylcoclaurine are phytochemicals found in embryos. Linalool, luteolineglucoside, dehydrnonaine, anonaine, armepavine, kaempferol-3-O—D-gluceronide, asimiloine, demethylco-claurine, and lirinidine are among the compounds found in the stamen. Quercetin, luteolin, luteolinglucoside, kaempferol, kaempferol-3-O-glucocide and isoquercitrin are present in the flower. The leaves contain roemerine, nuciferine, nornuciferine, armepavine, pronuciferine, N-nornuciferine, anonaine, liriodenine, quercetin, tartaric acid, gluconic acid, acetic acid, malic acid, ginnol, nonadecane, and succinic acid whereas the seeds contain dauricine, nuciferine, roemerine, lotusine, armepavine, liensinine, neferine, and isoliensinine[4,7].

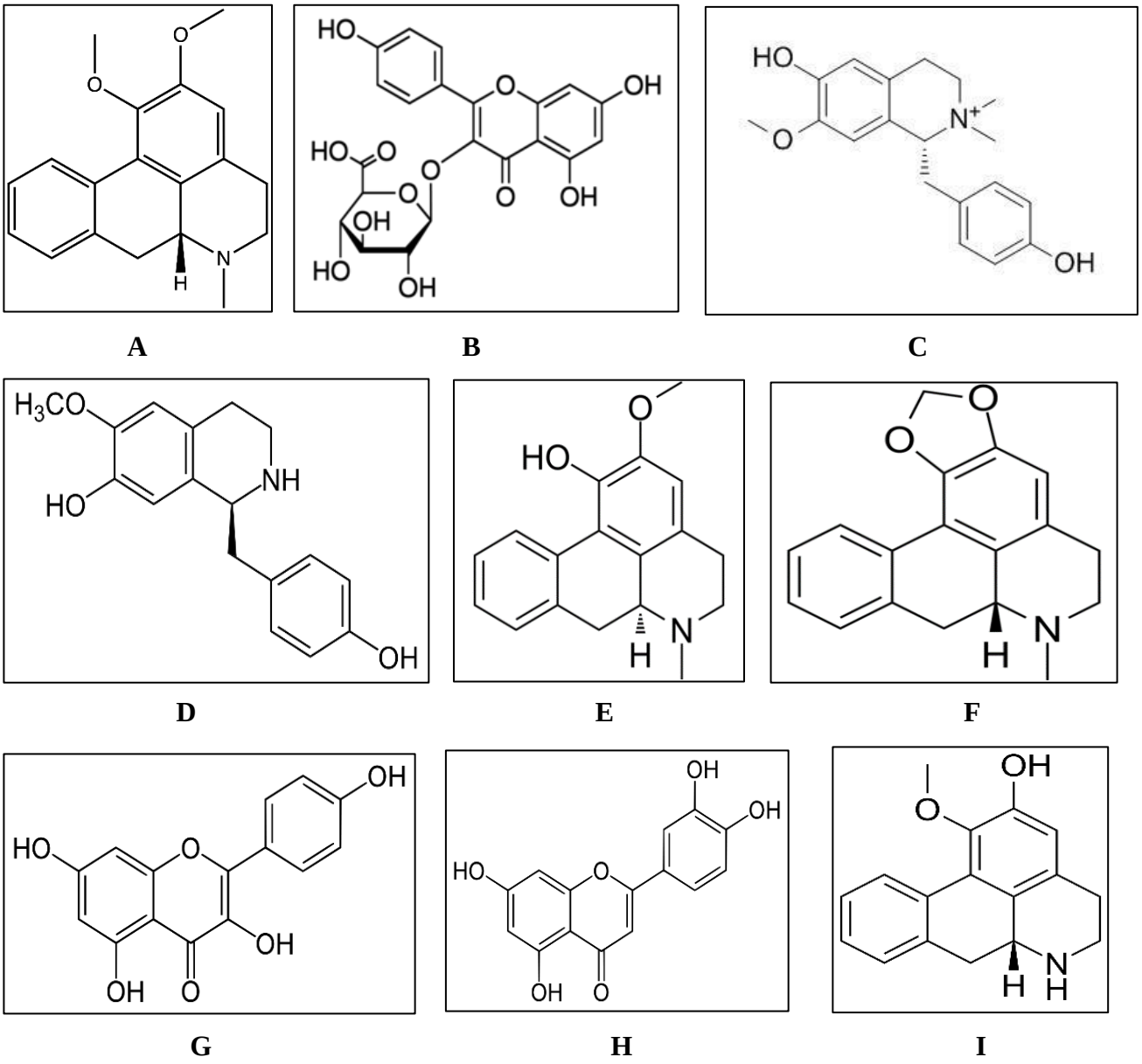

Figure 2: Chemical constituents present in *Nelumbo nucifera* (A: Nuciferine, B: Lotusine, C: Demethylcoclaurine, D: Kaempferol-3-O-*β*-D-glucuronide, E: Lirinidine, F: Roemerine, G: Kaempferol, H: Luteolin, I: Asimilobine)[8]

Antioxidant activity of Nelumbo nucifera

Owing to various metabolic activities or illnesses, free radicals, or reactive oxygen species (ROS) are produced in the cells of an organism. To prevent or neutralize the free radicals produced by these processes, exogenous and endogenous defensive mechanisms exist. Catalase, superoxide dismutase, and glutathione peroxidase are examples of endogenous sources, while vitamin C and E, as well as beta carotene, are examples of exogenous sources. Antioxidants are important to maintain the body's natural oxidant balance.[9]

Natural antioxidants are of particular interest to researchers due to the potentially harmful effects of synthetic antioxidants. Secondary metabolites include carotenoids, flavonoids, cinnamic acid, folic acid, ascorbic acid, tocopherols, and other phytochemical antioxidants[10].

Various in vitro and in vivo methods have been used to estimate the antioxidant properties of various parts of the lotus until now. However, no single metric is thought to be sufficient in determining total antioxidant capacity[11].

Antioxidant activity of N. nucifera leaf

According to in vitro assays, the methanol extracts of *N. nucifera* are observed to have free radical scavenging activity, metal-binding ability and reducing power, which may explain in part the mechanism behind the ability of the *N. nucifera* extract to protect cells from oxidative damage. Furthermore, the extract has concentration-dependent antioxidant properties against hemoglobin-induced linoleic acid peroxidation and Fenton reaction-mediated plasmid DNA oxidation[12]. Rutin, catechin, sinapic acid, chlorogenic acid, syringic acid, and quercetin, as well as a high total phenolic and flavonoid content, are found in the phenolic rich ethyl acetate fraction (EAF) from lotus leaves. It inhibits polyunsaturated fatty acid lipid peroxidation in a linoleic acid emulsion and shows strong reducing power against DPPH and ABTS cation radicals. It has also been shown to protect DNA from hydroxyl radical damage, as evidenced by the conversion of supercoiled pBR322 plasmid DNA to the open circular form. EAF also inhibits intracellular ROS formation and membrane lipid peroxidation in cultured hepatocytes, making it a cytoprotective agent against oxidative stress. Furthermore, EAF treatment significantly restored glutathione depletion caused by oxidative stress[13].

Methanol and Butanol extracts are found to have a higher capacity to scavenge DPPH radicals, delay LDL oxidation, and contain more antioxidative compounds. Quercetin and its glycosides inhibit LDL oxidation effectively, whereas myricetin-3-O-glucopyranoside has a stronger DPPH scavenging activity than the other flavonoids. These findings suggest that flavonoids play a role in the antioxidant capacity of lotus leaves[14].

Antioxidant activity of N. nucifera stamens

Stamens, which are necessary for reproductive functions, are also promising new sources of antioxidants. The ability of *Nelumbo nucifera* stamens to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and inhibit total ROS generation in kidney homogenates using 2',7'-dichlorodihydrofluorescein diacetate (DCHF-DA) and scavenge authentic peroxynitrites (ONOO-) has been investigated where EtOAc soluble fraction showed strong antioxidant activity after fractionation with several organic solvents, including dichloromethane (CH2CI2), ethyl acetate (EtOAc), and n-butanol (n-BuOH). Kaempferol had the highest antioxidant activity of the flavonoids isolated from the stamens. In the DPPH and ONOO- tests, the compounds kaempferol 3-O-13-D-glucuronopyranosyl methylester and kaempferol 3-O-13-D-glucuronopyranoside showed scavenging activity, whereas kaempferol 3-O-I-D-glucopyranoside and kaempferol 3-O-13-D-galactopyranoside were only active in the ONOO- test. Compound 13-sitosterol glucopyranoside, on the other hand, lacked antioxidant properties[15]. Other than flavonoids, the compound isorhamnetin isolated from *N. nucifera* stamens also show marked antioxidant properties in the DPPH and ONOO- assays[16].

Antioxidant activity of N. nucifera seeds

Apart from the alkaloids found in lotus seed embryos, flavonoids have received a lot of attention due to their potential health benefits in the treatment of diseases such as neurodegenerative diseases, type 2 diabetes, and cardiovascular diseases[17].

The flavonoid C-glycosides in lotus seed embryos were first discovered using tandem mass spectrometry and high-performance liquid chromatography (HPLC-MS)[18].

The antioxidant properties of lotus seed extracts have been investigated, as well as their effect on DNA damage in human lymphocytes. The findings revealed that boiling water extracts of lotus seeds (WELS) had higher antioxidant activity and extract

yields than other organic solvents. On ferrous ions, the WELS showed significant chelating binding and strong interaction with hydrogen peroxide. The antioxidant activity of WELS is thought to be due to phenolic acids such as caffeic acid, chlorogenic acid, p-hydroxybenzoic acid, gallic acid, and large amounts of phenolic compounds found in WELS. In human lymphocytes, the WELS revealed no changes in lipid peroxidation or DNA damage, regardless of whether hydrogen peroxide was used to induce them[19].

In vivo and *in vitro* studies on the antioxidant activity of a hydroalcoholic extract of *Nelumbo nucifera* seeds (HANN) confirm that it has strong free radical scavenging activity, as evidenced by low IC50 values in both the DPPH and nitric oxide methods. The values were discovered to be lower than the rutin standard. When Wistar rats were given HANN at 100 and 200 mg/kg body weight for four days before carbon tetrachloride (CCl4) treatment, there was a significant dose-dependent increase in superoxide dismutase (SOD) and catalase, as well as a significant decrease in thiobarbituric acid reactive substances (TBARS) in both the liver and kidney, when compared to CCl4-treated controls. The changes seen at 100 mg/kg bodyweight treatment were comparable to those seen at 50 mg/kg treatment with standard Vitamin E. The findings support HANN's antioxidant properties[20].

Comparative antioxidant properties of different parts of Nelumbo nucifera

On testing, ten different parts of the lotus and comparing in vitro for antioxidant activity, it has been observed that the receptacle not only had the highest phenolic, flavonoid, and proanthocyanidin content of the ten lotus extracts, but its DPPH and ABTS radical scavenging activities, reducing power, and total antioxidant activity was also comparable to, if not better than, the butylated hydroxytoluene (BHT) control. The metal chelating activity of all ten lotus extracts was significantly higher than that of BHT. Nonetheless, the ability of all ten lotus extracts to scavenge hydroxyl radicals was significantly lower than that of the ascorbic acid control. In contrast to their ability to chelate metals, the phenolic compounds in the ten lotus extracts are most likely responsible for their DPPH and ABTS radical scavenging abilities[21].

Through free radical scavenging activity, total phenolic and flavonoid content, and concentration of several specific flavonoids and alkaloids in the ethanol extracts of lotus seeds and rhizomes, the variations in antioxidant activity and concentration of functional components in the ethanol extracts of lotus seeds and rhizomes based on the growing region and dryness were investigated. Antioxidant activity and total phenolic content differed significantly depending on the growing region and dryness. The ethanol extracts of lotus seeds from Vietnam (Ho Chi Minh City), raw rhizomes from Korea (Siheung), and dried rhizomes from Japan (Nigata) had the highest specific flavonoid content, according to high-performance liquid chromatography analysis. The highest specific alkaloid content was found in ethanol extracts of seeds from China (Hubei), raw rhizomes from Japan (Nigata), and dried rhizomes from Korea (Siheung). In this study, astragalin, rutin, isoquercetin, nuciferine, dauricine, isoliensinine, and neferine were discovered for the first time in lotus rhizomes[22].

Discussion

The antioxidant activities of various organic and aqueous extracts of *N. nucifera* have been demonstrated in pharmacological studies. Researchers have made progress in recent years in characterizing the biological activities of lotus plant components, identifying some active components, and elucidating the mechanism underlying the antioxidant effects.

However, because of the high nutritional value and multiple active ingredients found in lotus, there is still a lot of room for research and development, as evidenced by several factors: The extraction, separation, and purification of lotus nutrients and bioactive components are still incomplete, with the crude extract still containing most of the components. To develop more effective extraction

methods in the future, a variety of separation methods and coordination optimization strategies for different components should be implemented. Similarly, the therapeutic efficacy of active ingredients from the lotus plant is still unknown; the mechanism has not been thoroughly investigated, and most studies have focused on solvent extraction. Separation, purification, and structure determination are difficult to achieve due to a lack of systematic research. As a result, further research into the fine structures of lotus active ingredients, as well as structure-activity relationship mechanisms, will be required in the future to overcome the challenges; traditional medicine studies have described many functions for the lotus plant; however, current research focuses on only a few fields. As a result, all the benefits of this plant parts are still purely empirical, with little or no scientific backing; future research should broaden the scope of lotus seed application and expand its biological and pharmacological properties. People are paying more attention to their health as their living standards rise. Consumers will increasingly recognize and prefer natural products as nutritious and edible efficacious drugs.

Conclusion

Lotus is widely used in pharmaceuticals and health care products. The antioxidant potential of different parts of the *N. nucifera* plant could be exploited for therapeutic applications. Deeper research and development of new functional foods derived from the lotus plant will broaden the nutrition characteristics and physiological activities for a deeper processing pathway, resulting in significant economic and social benefits, as well as further exploitation in human health improvement.

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Effect of *Allium sativum* on Cardiovascular Consequence as Immunomodulator in Post Covid 19: A Review

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Abstract

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is a novel coronavirus under the family Coronaviridae. COVID-19 has now spread globally with increasing amount of infected and death among all populations. COVID-19 disease develops after average incubation period of 4 days (2–7 days) following infection by SARS-CoV-2. Cardiac complications lean towards to occur 8–14 days after the viral infection. COVID-19 patients have commonly developed with coagulation disorder and fibrinolytic dysfunction even after the recovery. According to the Unani system of medicine, enhancing immunity with immune boosters is one of the main aims for preventing the disease and maintaining the health. Garlic (*Allium sativum*) is measured as a good plant source for maintaining the homeostasis of the immune system. From the ancient period, garlic is being used for treatment of cardiovascular diseases. The aim of this review was to provide modernized, wide-ranging and considered information on post COVID-19 cardiovascular consequences and the effects of garlic on cardiovascular consequences as an immunomodulator. Systematic literature searches were conducted on MEDLINE, PubMed, Science direct, Springer databases and popular search engines were included. The results gained garlic possesses hyperlipidemic, antithrombotic, anti-atherosclerotic properties. In addition to that it has a broad range of therapeutic effect from such as antibacterial to anti-cancer. Summary was formulated after a thorough reading of all reading materials. This review was concluded that garlic acts as an immunomodulator and minimize the consequences of cardiovascular ailments.

Keywords: COVID-19, Cardiovascular ailments, Immunomodulator, Fibrinolytic, *Allium sativum*

Introduction

Overview of COVID-19 and its effects on cardiovascular diseases

The Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) is a novel coronavirus under the family Coronaviridae. It is the seventh human coronavirus and identified to be the responsible for the current epidemic. It was originated as acute atypical respiratory infections in Wuhan, China in early December 2019[1]. The disease caused by this virus, termed coronavirus disease 19 (COVID-19). It has rapidly spread throughout the world within a short period with increasing rates of morbidity and mortality. This has led to a pandemic emergency, and it has been declared by the WHO on March 11, 2020[2]. COVID-19 disease develops after average incubation period of 4 days (2–7 days)[3] following infection by SARS-CoV-2 virus. Cardiac complications lean towards to occur 8–14 days after the onset of the viral infection and predict poor prospects. Although the real implication of this is mysterious, as our understanding of the disease is improving, cardiac complications are being increasingly predictable[3].

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A study done on recently recovered patients from a German cohort revealed that cardiac involvement was present in 78% and ongoing myocardial inflammation in 60% of the patients[3].

COVID-19 patients commonly develop with coagulation disorder and fibrinolytic dysfunction even after recovery. Disseminated intravascular coagulation (DIC) is most commonly seen in COVID-19 infected persons. SARS-CoV-2 infection causes immune insufficiency, injuries in the endothelium, and activation of the platelet, and inflammatory mediators release (IL-6 and TNF-α), causing a procoagulant state. DIC can contribute to the injury of the myocardium causes microthrombus. The SARS-CoV-2 infection has allied to the harm of the endothelium, resulting in the huge spectrum of organ involvement (renal disease, thrombosis and pulmonary embolism, and cerebrovascular damages). The rates of thrombotic problems documented in the reviews were highly variable. Deep vein thrombosis, pulmonary embolism, and acute cor-pulmonale have been identified. Previous literatures recommended the use of pharmacological venous thromboembolism prophylaxis for hospitalized Covid-19 patients[3,4].

The Unani system of medicine explains to enhance immunity by using immune boosters is one of the main things for preventing disease and maintaining of health. Therefore, an approach to enhance immunity and provide a symptomatic cure in upper respiratory tract infection is advocated[2]. As per the saying "Prevention is better than cure", the Unani system of medicine has found more measures with diet and medicines. Garlic is measured as a good plant source for maintaining the homeostasis of the immune system. Thus, different reviews have established exciting advantageous effects of garlic on the immunity and immune cells[5]. Garlic has also been stated to protect from epidemic diseases. Plenty of studies exposed garlic to have antioxidant, anti-inflammatory, immune-modulating, antibiotic, bacteriostatic, antifungal, antiviral, anthelminthic, antithrombic, hypotensive, hypoglycemic, and hypocholesterolemia properties[6].

The purpose of this review is to provide modernized, comprehensive, and categorized information of post COVID-19 cardiovascular consequences and the effects of Allium sativum on cardiovascular consequences as an immunomodulator.

Materials and Methods

Systematic literature searches were conducted on Google scholar, MEDLINE, PubMed, Science Direct, Springer databases, and popular search engines were included to collect the necessary information. A huge number of recently published research papers were studied during the year 2020-2021 to collect the data about the effects and consequences of COVID-19 and garlic. In addition to those some medicinal plant hand books were reviewed to collect data about garlic. Data extraction was done by using the keywords as Allium sativum, garlic, allium, cardiovascular diseases, COVID-19 infection, antifibrinolytics, ant thrombolytics, immunomodulators.

Data arrangement

The results were construed and categorized on the basis of an application to the subtopics and, a summary of all effects was stated Each subtopic states a brief review of the plant and the information is supported by the results of various pharmacological studies conducted in that field. Finally, a conclusion was reached based on the reviewed information. The summary was done after a thorough reading of all reading materials.

Discussion

From the ancient period garlic is being used for the treatment of cardiovascular ailments. A systematic literature study shows in taking garlic has a significant effect on minimizing blood pressure, prevention of atherosclerosis, lowering of serum cholesterol and triglyceride, inhibition of platelet aggregation, and increasing fibrinolytic activity[7].

Allium sativum

Garlic, *Allium sativum* L. is a plant under the Liliaceae family, has been broadly recognized as a valued spice and a common remedy for various diseases and functional disorders[8]. Garlic, the name

may have initiated from the word 'all' meaning pungent. Sanskrit histories reveal its medicinal values dating back to 5000 years, and it has been used for a minimum of 3000 years in Chinese medicine. The Egyptians, Babylonians, Greeks, and Romans used garlic for healing diseases. In 1858, Pasteur pointed garlic's antibacterial property, and during World War I and World War II it was used as an antiseptic to stop gangrene[9]. Garlic currently used to prevent and cure cardiovascular disease by lowering blood pressure and cholesterol. It also prevents platelet aggregation, improves fibrinolytic activity, and reduces clots on injured endothelium. It also used as an antimicrobial agent, and as a preventive agent for cancer[9].

Classification of Allium sativum

Kingdom: Plantae, Subkingdom: Tracheobionta, Order: Asparagales, Family: Amaryllidaceae, Genus: Allium [10].

Vernacular Names

Sanskrit: *Lasuna*, *Rosona*, *Yovanesta*. English: Garlic, poorman's treacle Bangali: *Rosun*, Hindi: *Lashan*, *lahsun*. Arabic: *SaunTaum* German: *Knoblauch*, *Lauch*. Greek: *Allidion*, *Skorodon*. Italian: *Aglio*. Chinese: *Syuntauh*. Urdu: *Lehsun*. Malayalam: *Veluthull* [10].

Chemical constituents

Garlic contains 65% of water and it makes the highest contentand the remaining portion of the dry weight composed with fructose-containing carbohydrates, compounds of sulphur, proteins, fibers, several enzymes, and free amino acids. In addition, it contains high amount of potassium, zinc, sulphur, moderate amount of selenium, and low amount of calcium, magnesium, sodium, iron, and manganese. It also contains Vitamins A, C, and B-complex. Seventeen types of amino acids found in garlic; lysine, histidine, arginine, aspartic acid, threonine, swine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan, and phenylalanine. It contains at least 33 sulphur compounds such as alliin, allicin, ajoene, diallyltrisulfide (DATS), S-allyl cysteine, vinyl dithiines, allyl propyl disulfide, S-allylmercaptocystein. Garlic's characteristic pungent odor is produced from these Sulphur compounds. The most abundant compound of garlic is alliin it is relatively odorless. Garlic's characteristic pungent odor is present at 10 and 30 mg/g in fresh and dry garlic respectively. Phenolic compounds such as Caffeic acid & Ferulic acid are also contained in garlic in high numbers. During cutting or crushing an enzyme called alliinase comes in contact with alliin and produces allicin. The principal bioactive compound, Allicin (diallylthiosulfinate) is present in the aqueous extract of garlic or fresh garlic homogenate, which gives off off garlic's distinct characteristic fragrance. The compounds present in garlic are water-soluble [(97%) SAC(S-allylcystein), NAC(N-acetylcystein)] and small amounts of oil-soluble compounds [(0.15-0.7%) DATS (diallyl disulfide), DAS (diallyl sulfide)][7,11,12].

Pharmacological activities

Nature's one of the wonder plants is garlic and it has strong healing properties. It can stop the growth and kill micro-organisms. Also, it lowers blood pressure, blood cholesterol, blood sugar, and prevents blood clotting. Especially it contains anti-cancer properties. It can also enhance the immune system and protect health.

Compounds which are present in garlic which have the power to prevent the human body from a wide variety of diseases[12]. Such as hypertension, dyslipidemia, coronary heart disease, myocardial infarction, and atherosclerosis are being treated by garlic. Some types of cancers are also being treated and prevented with garlic and its compounds; colon cancer, rectal cancer, stomach cancer, breast cancer, prostate cancer, bladder cancer, and lung cancer. It is also used to cure enlarged prostate (benign prostatic hyperplasia - BPH). Diabetes mellitus, knee joint pain, allergic rhinitis, diarrhea, high blood pressure late in pregnancy (pre-eclampsia), common cold, and flu also could be treated with garlic. It is used to enhance the immune system, curing tick bites, and preventing and treating various types of infections [10]. Garlic is very useful to treat fever by reducing

temperature, productive coughs, chronic headache, stomach ache diseases, sinusitis, gouty arthritis, rheumatism, piles, asthma, chronic bronchitis, hypotension, hypo and hyperglycemia, and snakebites. It is also used to cure stress and fatigue, and maintaining healthy liver function. Further, garlic useful in the conditions of arthritis, sciatica, lower back pain, tuberculosis, malaria, several types of skin diseases including leprosy, vitiligo, discoloration of the skin, and itches. Garlic plays a great role in indigestion, colic pain, splenomegaly, fistula, bone fracture, kidney stone, anemia, jaundice, epilepsy, cataract, and night blindness. Garlic paly huge role in the area of pharmaceutical science [13].

Stimulant, antiseptic, anthelminthic, antihyper-tensive, carminative, diaphoretic, expectorant, diuretic, antiscorbutic, aphrodisiac, and anti-asthmatic properties have been recorded for garlic and its compounds. It is used for the relief of rheumatic pains. *Allium sativum* leaf lectin (ASAL) protein is a compound in garlic and that helps the blood to flow more freely and reduce the incidence of clots. A daily dose of 1 mL/kg body weight of garlic extract for six months can result in a substantial reduction in free radical stress in the blood of patients with atherosclerosis and cholesterol circulating in the bloodstream. Oxidation reactions are prevented by garlic may explain some of its advantageous effects in atherosclerotic cardiovascular diseases[14].

Hypolipidemic, antithrombotic, and anti-atherosclerotic properties of garlic were documented. In addition to that, a broad range of therapeutic effects such as antibacterial, immunomodulatory and anticancer effects also have been included[15].

Immunomodulatory effects of garlic

Garlic produces immunomodulatory properties with its contents. Antiviral activity of the garlic extract against influenza B, herpes simplex, and coxsackieviruses were documented. In this review, the highest virucidal activity was observed in ajoene followed by allicin, allyl-methyl thiosulphinate, and lastly methyl-allylthiosulphinate. Its action against cytomegalovirus and Avian infectious bronchitis virus are also identified later. It has also been detected to stimulate macrophages and encourage immunoglobulins to enhance immunity. Specific to the respiratory system, garlic was methodically considered to improve lung function in heavy smokers. Its consequence in the common cold was found evidently by some researchers as reducing the quality and quantity of the exudates. Efficacy of garlic extract in asthma-like condition of lungs as examined and verified that it has immunomodulatory properties[6].

Sulfur-containing amino acids and other compounds are contained in garlic that appears to increase the activity of the immune system. It is one of the remarkable conductors of the body's immune system; which arouses immune function by making macrophages or killer cells more energetic. People are regularly affected by insufficient nutrition, smoking habit, physical damage, psychological impact, and chemical pollution.

Its content of germanium alone provides admirable immune stimulation. In addition to germanium, garlic contains thiamine, sulfur, niacin, phosphorous, and selenium[8].

In order to fight with the infectious diseases, additional nutrients like garlic are obviously needed to boost our immune system.

Superior immunomodulatory effects are shown in aged garlic extract (AGE) than raw garlic; these effects are ascribed to the converted organosulfur compounds. Recent reviews have shown that the extract of aged garlic also contains some immunomodulatory proteins (SAC(S-allylcystein), SAMC (S-allylmercaptocysteine) such as immunoglobulin which have been identified as the major garlic proteins or agglutinins. Further, the results suggest that immunomodulatory proteins and fructans contribute to the therapeutic potential of extract of aged garlic, in addition to the crucial transformed organosulfur compounds[14].

Garlic acts as an immunity booster. It can be used in respiratory tract infections such as common cold, allergic rhinitis, asthma, and acute and chronic bronchitis. Studies have proven the binding modes of its chemical constituents for natural remedies of garlic against COVID-19[24].

Cardio protective activity of garlic

Garlic is a popular supplement considered a healthy choice for people who are looking to increase cardiovascular wellness. Approximately 4% of all cardiovascular disease patients and 30% of cardiovascular patients who use herbal supplements to treat cardiovascular ailments used to take garlic in their remedies. Indeed, as early as the 1920s and 1930s, numerous studies do bear beneficial cardiovascular effects of garlic. Garlic reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA. It has also been shown to inhibit LDL oxidation, platelet aggregation, arterial plaque formation, decrease homocysteine, lowers blood pressure, and increase microcirculation, which can helps in prevent diabetes, heart disease and dementia[10].

Both experimental and clinical studies on garlic and its different preparations demonstrate significant effects on cardiovascular diseases (CVDs) that includes lowering blood pressure, prevention of atherosclerosis, reduction of serum cholesterol and triglyceride, inhibition of platelet, aggregation, and increasing fibrinolytic activity. These favorable cardiovascular effects of garlic decrease the risk of peripheral arterial occlusive diseases, plasma viscosity, and unstable angina and increase the elasticity of blood vessels and capillary perfusion. Daily in taking of cloves of raw garlic bulb for 6 months shows an 80% reduction of serum thromboxane B2 in middle-aged men. In situ study in a rat model, showed that an overall antithrombotic effect of garlic due to modulation of fibrinolytic activity through increased plasminogen activation and thrombin inhibition[7,12,17,18]. Garlic and its preparations have been widely known for its preventive and treatment purposes of many cardiovascular diseases (CVDs) that include atherosclerosis (narrowing of the blood vessels), hyperlipidemia, thrombosis, and hypertension[12].

The positive effect of garlic on the circulatory system is extremely well documented and it has been proved to; lower blood pressure decreases platelet aggregation and lower serum triglycerides and LDL-cholesterol levels increase serum HDL-cholesterol and the process through which the body breaks up blood clots. And also, it stimulates the production of nitric oxide in the inside layer of blood vessel walls, a substance that helps them to relax. As a result of these beneficial actions, garlic helps to prevent arteriosclerosis and thereby reduces the risk of heart attack or stroke. Two or three cloves a day have cut the risk of subsequent heart attacks in half of the heart patients. Garlic's ability to reduce the number of free radicals in the bloodstream may be the reason for these beneficial effects of garlic[8].

Garlic and its derivatives have proven inhibition of platelet aggregation in vitro and in animals and reduction of platelet-dependent thrombus formation. Anti-platelet activity may be attributable to garlic constituents including adenosine, allicin, and paraffinic polysulfides19. Fibrinolytic activity has found that garlic increased fibrinolytic activity in healthy individuals as well as in acute myocardial infarction patients[20].

Platelet aggregation is a leading cause of thrombosis and myocardial infarction. The prostaglandin and prostacyclin produced by endothelial cells lining the blood vessels prevent adherence to the blood vessels. Therefore, disc-shaped blood platelets come into contact with foreign surfaces, collagen in the injured vessels wall, and form plaques that inhibit the flow of blood. This process is platelet activation. The stimulated platelets alternate their shape, put out pseudopodia, release their granules, and stick to other platelets, beginning the process of platelet aggregation. Studies have shown that fresh garlic, garlic powder, and garlic oil have great potential in inhibiting platelet aggregation. Several garlic compounds contribute to the anti-thrombotic effect. These effects appear to be important contributors to garlic's beneficial effects in atherosclerotic conditions[21,22].

Allicin is the main bioactive compound found in garlic which has beneficial effects on the cardiovascular system. From the pharmacokinetic reviews, allicin is known to be aquaphobic and can be willingly absorbed through the cell membrane without encouraging harm to the phospholipid bilayer and then rapidly absorbed to exert pharmacological

properties that are important to the cardiovascular system. It was found to provide cardio-protective effects by inducing vasorelaxation and alleviating various pathological conditions of the cardiovascular system, including cardiac hypertrophy, angiogenesis, platelet aggregation, hyperlipidemia, and hyperglycemia[23].

Conclusion

Since ancient period, garlic (Allium sativum. L) has been described to various pathological conditions. Biological active constituents of garlic contribute an energetic role in various disease conditions including COVID-19 infection. Furthermore, the study revealed the potential pharmacological activities against cardiovascular diseases and its consequences after COVID-19 infection. Garlic produces immunomodulatory properties with its contents and acts as an immunity booster. This review was concluded as Allium sativum acts as an immunomodulator and to minimize the consequences of cardiovascular ailments.

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