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## Cover story

### *Maha Daluk*

***Euphorbia neriifolia* Linn.**
**Family: EUPHORBIACEAE**
**Vernacular names: Sinhala:** *Ma Daluk, Maha Daluk, Kola Pathok*; **Sanskrit:** *Snuhi, Vajraduma, Guda, Nagarika, Nanda, Nistrinsapatra, Patrasnuhi;* **English:** Indian Spurge Tree, Common milk hedge; **Tamil:** *Ilaikalli, Perumbu Kalli* **Hindi:** *Sehund, Danda thukar*

Plant shown in the cover page is *Euphorbia neriifolia* Linn. It is a large, glabrous, fleshy, erect shrub or small tree approximately 1.8-4.5 m in height. It has saccular branches having a pair of strong stipular spines on spirally arranged tubercles. The young leaves are dark green in color, having a leathery texture and a reticulate venation. The flowers are yellowish green in colour. Male and female flowers occur concurrently inside the same bunch. Fruits are looking like capsule. Style 3-fid, stigmas slightly dilated and minutely toothed. Seeds are flat containing soft hairs. Latex is a milky sap-like fluid.

*Euphorbia neriifolia* grows in dry, rocky hill areas of South Asia; found and cultivated in India, Sri Lanka, Burma, Bangladesh, Thailand and Malaysia. Ethnomedicinal uses of latex, leaves, roots and whole plant of *E. neriifolia* are documented. The latex of *E. neriifolia* is used in Ayurvedic formulations like *Avittoladi bhasma*, *Jatyadi varti*, *Snuhi ghrta* and *Jalodarari rasa*. This plant is useful in abdominal troubles, bronchitis, tumors, loss of consciousness, asthma, leucoderma, piles, inflammation, enlargement of spleen and flatulence etc. Latex is also famous as an ingredient for preparation of *Kshara Sutra* used for treating sinuses and fistula in ano. Externally latex and juice of leaves are applied for earache, ulcers, warts, scabies and to prevent suppuration.

This plant has the phytoconstituents such as flavonoids, monoterpenoids, diterpenoids, triterpenoids, and alkaloids. The plant consists of proven anti-inflammatory, anti-carcinogenic, antidiabetic, antiarthritic, anticonvulsant, and antioxidant properties that can be attributable to its phytochemical profile. The latex of the plant is toxic and it can cause skin and eye irritation with intense inflammation. Therefore, the processing and use of raw materials should be done with precautions. In Sri Lanka traditionally *Daluk* (*Euphorbia antiquorum*) is used in place of *Snuhi* while we have the same plant species in the country.

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***Cover story and Photograph by Dr. B.M.S. Amarajeewa***
***Cover page designed by Mr. K.K.P.R.K. Kohombakanda***

# Sri Lanka Journal of Indigenous Medicine (SLJIM)

**Volume 07 Number 02 Page 623 - 666 December 2022**

## Contents

**Page No.**

**Case study**

Management of Lymphoedema by *Varma* therapy: A case study 623
*Anpuchelvy S., Sritharan G. and Ganesan S.*

**Experimental study**

Antimicrobial activity and phytochemical analysis of *Rivea ornate* 630
*Karunarathna A., Jeyadevan J. and Thavaranjit A.*

Protective activity of *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. 637
and *Withania somnifera* (L.) Dunal against lipid peroxidation, protein oxidation
and deoxyribose oxidation
*Wijesekara M. A., Soysa P., Jayasena, S., Kottahachchi D. U., Perera D,*
*Jayasiri A.P.A., Wimalachandra M. and Gooneratne L. V.*

**Review article**

In-silico and *in vitro* evidence of anti-dengue viral action in a selected Sri Lankan 645
medicinal plants; A narrative review
*Gunasekera K.M.*

Dissemination of knowledge for health and wellbeing: with special reference 658
to Buddhism and Ayurveda
*Gamage C. K.*

# Management of Lymphoedema by *Varma* therapy: A case study

**Anpuchelvy S.[1*], Sritharan G.[2] and Ganesan S.[3]**

**Abstract**

*Varmam* is the flow of vital energy in the body. Energy junctions in the body are called *Varma* points. Proper vibration or turning of the vital points (*Varmam*) by experts promotes health. These are very important vital places and any injury to these parts may lead to complications. In this case study, 22 years old female patient with lymphoedema and pain in both legs was selected. Finally identifying the *Varma* points and *Varma* stimulation therapy was done three times daily for 14 days and heated *Thalankai* oil-soaked cotton was applied over stimulated regions. 5th-day *Patthu* application and 13th-day, *Suddigai (Agni*) *karma* therapy were done on *Varma* points. After fourteen days the patient shows relief from the symptoms she suffered. This case study can be considered evidence of *Varma* therapy for lymphoedema. This can be explained by the concept of *Varma* stimulation on *Varma* points which leads stimulation to the endocrine system *Anagatham* (Thymus gland) and activate the Lymphatic channels. Lymphedema occurs when the lymph system is damaged or blocked. Fluid builds up in soft body tissue and causes swelling. So *Anagatha chakra* activates the Lymphatic channels and that effect plays a direct part in lymphoedema. The most important outcome of the study is relieving the symptoms and there is no recurrence within the study duration, as well as the follow-up, was done every month for more than eighteen months. Hence this study has a positive outcome and can be recommended as the therapeutic procedure for Lymphoedema.

**Keywords:** *Varma, Marma, Thalankai oil, Anagatha chakra*, Lymphoedema

## Introduction

*Varmam* is one of the basic Siddha medical treatment because *Tridosha* is rectified only with the help of *Varma.* It is said that the 1st Siddhar Lord Shiva taught *Varma* to his son Murugan. Murugan saint to Agasthiyar and Agasthiyar in turn to his disciples. Agasthiyar was the prime and pioneer of all Siddhars. He was also called "*Kumbamuni*". *Varmam* is a vital energy flow circulating inside the body[1]. It is the manifestation of the basic five elements (water, earth, air, space and fire), three bio -humours (*Vata*, *Pitta* and *Kapha*), ten bio -energy transmitter pathways (*Naadi*), vital energy (*Vasi*) and *Kundalini*[2]. *Varma chikitsa* is very popular in many places as a traditional skill[3]. A number of bone setters and *Nadi vaidyas* practice by this specialty. But as a traditional skill, it has no scientific explanation behind it and it is limited to some traumatic lesions of muscle and bones. The *Varma* therapy can be done in an hour. This science is still obscure. In light of the theoretical description available in the old texts and present research and knowledge, *Varma chikitsa* has been further developed and practiced[4]. *Varma* therapy contributes to increasing or recharging physical, mental and spiritual energies. On the physical level, it helps to revitalize or reenergize the body tissues; at the cellular level, it improves vital functions like digestion, respiration, blood circulation and excretion[5]. On the psychological level, it improves the mental faculty by directing it in a positive direction. It also offers a way to treat many psychosomatic ailments without any drugs. It harmonizes the functioning of nervous and endocrine systems to control psychological

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disorders[6]. The aim of Siddha Ayurvedic medicine is to preserve the health of a healthy individual and to cure the diseases of the diseased person. There is a major role of *Varma* therapy and yoga along with Siddha and Ayurveda medicine to fulfill the above-mentioned goal. Under the present circumstance, many people have come to realize the importance of *Varma* therapy and *Yoga* as practical methods of improving the state of bodily health and the quality of life. *Varma* therapy and yoga are used for achieving equilibrium, harmony and balance in day-to-day life.

Lymphoedema is a chronic (long-term) condition that causes swelling in the body's tissues[7]. It can affect any part of the body but usually develops in the arms or legs. Other symptoms of lymphoedema can include an aching, heavy feeling in affected body parts and difficulty moving them[8]. The lymphatic system is a network of vessels that carry protein-rich lymph fluid throughout the body. It's part of your vessels that carry protein-rich lymph fluid throughout the body. It's part of your immune system. Lymph nodes act as filters and contain cells that fight infection.[9]

## Case study

A 22-year-old female patient with lymphoedema and pain in both legs is selected for the case study. The patient was very active, and plays golf each weekend, experienced swelling in her left knee and ankle joint and later in the right leg with severe pain and got her first treatment in 2019. The physician noted the patient's apparently lymphoedema and asked if she had ever been treated for it. Same time patient developed itching of the legs and was referred to a Dermatologist to treat the hyperpigmentation. Thereafter the patient was referred to a Rheumatologist in September 2020 but no improvement was found. Then referred to the surgical clinic for evaluating bilateral lower limb swelling in 2021. The Magnetic resonance venography (MRV) of the Bilateral lower limb study did not show any abnormality. Subcutaneous edema seen in both lower limbs could be due to lymphoedema. Knee joint effusion is seen on the left side. The patient was advised to do a bandage to reduce edema in her legs, but she felt difficult to practice, which lead to difficulty to reduce. Then the patient was referred to the treatment center for treatment and a home maintenance program. The patient seeks native therapy and presented to Herbal Health Care with the same complaints.

## Diagnosis

The diagnosis was done according to the evidence of Magnetic resonance venography (MRV) and ultra-scan reports of Bilateral lower limb impression of lower limbs. Knee joint effusion is seen on the left side. An assessment of both legs and feet indicated for bi- lateral lymphoedema.

## Treatment

### *Treatment regimen*

The patient is guided to sit in a comfortable position and adopted the main procedure (*Pradhana karma*).

### *Pradhana Karma*

Initially identifying the *Varma* points for the *Varma* manipulation method. The selected *Varma* points were *Panchamuka varmam, Komperikalam varmam, Kalcanni varmam, Ullankal vellai varmam, Muttu varmam, Muttukkannu varmam, Muttu cirattai varmam, Kutirai nuni nakku varmam* and *Kanapati mukav.* The *Varma* stimulation therapy was done daily for 14 days continuously.

### *Panchamuka varmam*

Location: Around the patella

Patient position: Supine position.

Physician approach: Reach the *Varmam* point from the front side of the patient.

Finger selection: both the thumb (Medial ¼ part of the thumb).

Procedure: place the tip of the thumb along the base of the patella and glide over the borders till the apex of patella (Figure 1, 2 and 3).

Duration: 30 seconds

Force type: ½ *Mathirai* (Frequency): 3 times

### *Komperikalam varmam*

Location: Eight finger breadths above the medial malleolus.

Physician approach: place the tip of the middle three fingers over the point. Press three times (in a pumping motion) towards the medial border of the tibia.

Duration: 30 seconds

Force type- ½ *Mathirai* (Frequency): 3 times

***Kalcanni adankal varmam***

Location: At the junction of the big and second toe.

Physician approach: Place the tip of the thumb over the *Varmam* point and then press and release

***Ullankal vellai varmam***

Location: At the junction of the big and second toe in the plantar region.

Physician approach: Place the tip of the thumb over the *Varmam* point and then press and release.

***Muttu varmam***

Location: Center of popliteal fossa.

Physician approach: Place the tip of the middle three fingers over the points, press and move upwards.

***Muttukkannu varmam***

Location: In the dimple just below the base of the patella on either side.

Physician approach: Place the finger on the *Varmam* point and press or give intermittent pressure. Also, stimulate *Muttu cirattai varmam.*

***Muttu cirattai varmam***

Location: In the base and apex of the patella.

Physician approach: Place the thumb on the *Varmam* point and press. Also, stimulate *Mutukkanu varmam* simultaneously.

***Kutirai nuninakku varmam***

Location: The lower end of the calf muscle (posterior aspect).

Physician approach: Place the thumb over the *Varmam* point and then press (Simultaneously the patient is asked to flex and extend the neck) (Figure 5 and 6).

***Kanapati muka varmam***

Location: Five-finger breadth above the *Kutirai muka varmam.* Directly opposite to *Kutirai adi nakku varmam* in the anterior aspect of the leg.

Physician approach: Place the thumb on the *Varmam* point and press for 30-60 seconds and give rotatory motion followed by stimulation in the posterior direction.

The *Varma* stimulation therapy was done daily for 14 days. After the *Varma* stimulation, heated *Thalankai* oil soaked with a cotton piece was applied over stimulated regions (Figure 7). 5$^{th}$-day *Pattu* application and 13$^{th}$ day (Figure 8) applied the *Suddigai (Agni) karma* therapy on each *Varma* point (Figure 9 and 10).

***Preparation of Drugs***

The oil of *Thalankai* was prepared according to the classical text of Siddha Ayurveda Ovdathasangiram[10]. Preparation of Traditional *Pattu* was done according to the methods mentioned in the "Jaffna traditional Siddha Remedies" by Dr. Ganesh.

***Traditional Paste***

*Withania somnifera* -1 part (60g), *Caryophyllus aromaticus*) -1/2 part (30g), *Syzygiumaromaticum* -1/2 part (30g), *Vigna mungo* -1 part (60g), were grinded with 150 ml of egg white and mixed with 100ml bee honey to a semi-solid foam paste.

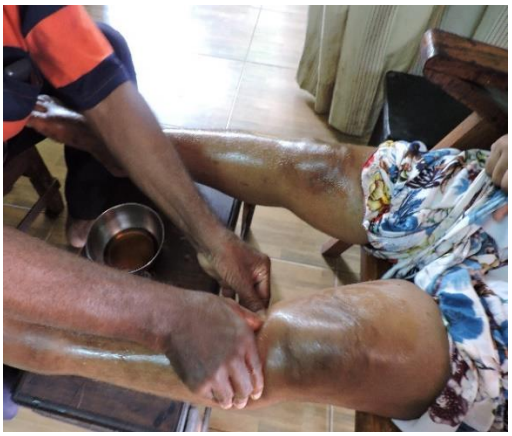

**Fig.1: *Varma* stimulation of the left knee joint *Panchamuga varmam***

**Fig. 2: *Varma* stimulation of the left knee joint *Panchamuga varmam***

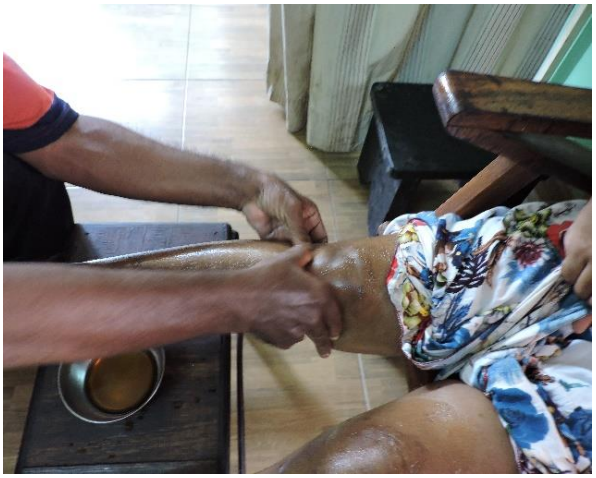

**Fig. 3: *Varma* stimulation of the right knee joint *Panchamuga varma***

**Fig. 4: *Varma* stimulation of the left ankle joint**

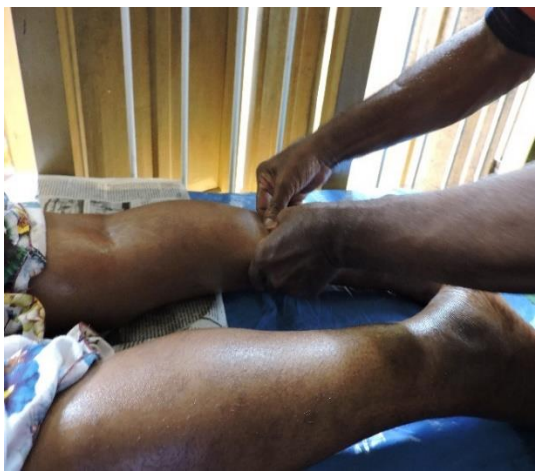

**Fig. 6: *Kuthirai nuninakku varma* stimulation (Left leg)**

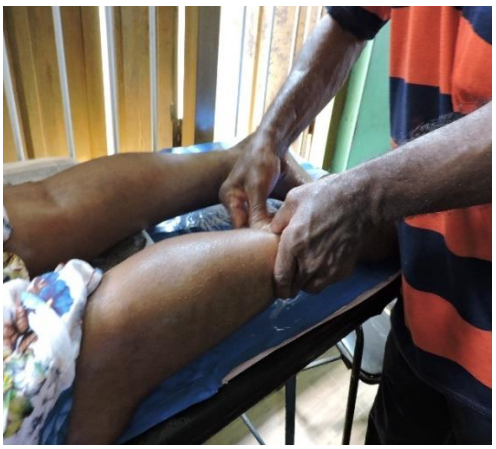

**Fig.5: *Kuthirai nuninakku varma* stimulation (Right leg)**

**Fig. 7: Heated medicated oil with cotton apply the affected part**

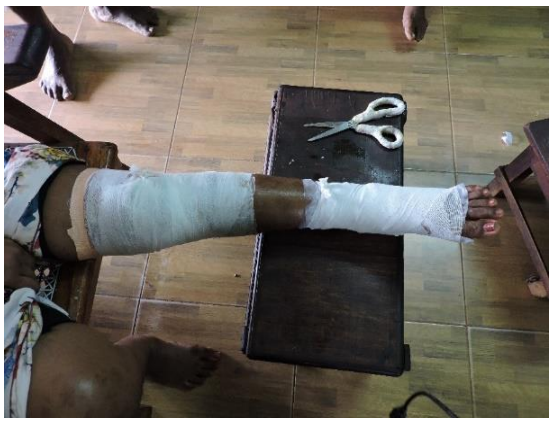

**Fig. 8: *Pattu* (Medicated poultice) application**

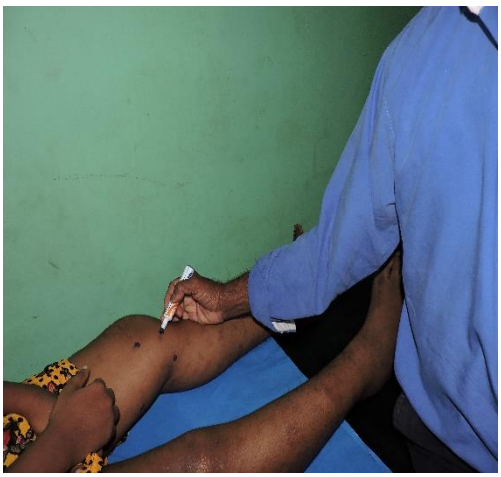

**Fig. 9: Identify the *Suddigai* point**

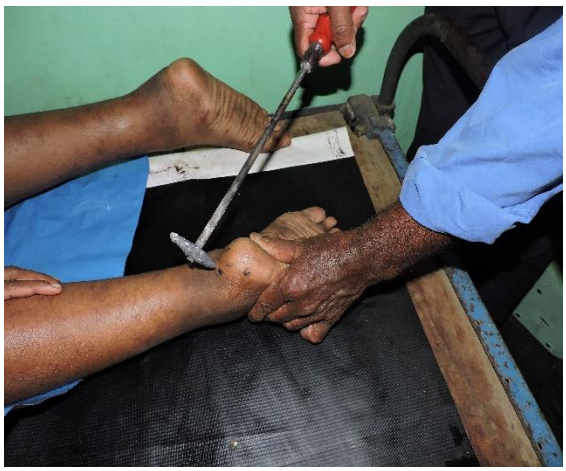

**Fig.10: Application of *Suddigai (Agnikarma)***

## Results

Table 1 shows the leg measurements before and after treatment

**Table 1: Leg Measurements (cm) before and after treatment**

| | Right leg (cm) | | Left leg (cm) | |
|---|---|---|---|---|
| | **Before treatment** | **After treatment** | **Before treatment** | **After treatment** |
| **Midpoint of the knee joint** | 38.2 | 36.2 | 39.8 | 36.4 |
| **Midpoint of the calf** | 41.9 | 39.3 | 43.2 | 39.5 |
| **Midpoint of the ankle joint** | 26.8 | 23.8 | 26.8 | 24.0 |

.

## Discussion

According to Agustheyar, there are 108 vital points mentioned in the body, which are called "*Varmam*." These are very important and vital places as any injury to these parts may lead to severe pain, disability, loss of function, loss of sensation (anesthesia) and death[11]. Meanwhile, eleven *Varma* points are mentioned in each leg region. In Siddha medicine, the concept of *Varmam (Marma)* plays a vital role as a disease affecting these vital parts has a bad prognosis[12]. Certainly, we can say that the disease or lesions away from the *Varmam* can be treated easily. When the *Varma* points, gets injured there can be a fatal response[13]. Keeping this concept in mind one should try to apply *Varma chikitsa* to provide the cure for different body ailments. *Varma* therapy contributes to increasing or recharge physical, mental and spiritual energies. On the physical level, it helps to revitalize or reenergize the body tissues; at the cellular level, it improves vital functions like digestion, respiration, blood circulation and excretion[14]. On the psychological level, it improves the mental faculty by directing it a positive direction. It also offers a way to treat many psychosomatic ailments without any drugs[15]. It harmonizes the functioning of nervous and endocrine systems to control psychological disorders. This case study can be considered as an evidence of *Varma* therapy for lymphoedema. This can be explained by the concept of *Varma* stimulation on *Varma* points which leads stimulation to the endocrine system Anagatham (Thymus gland) and activate the Lymphatic

channels. Lymphoedema occurs when the lymph system is damaged or blocked. Fluid builds up in soft body tissue and causes swelling. So *Anagatha chakra* activates the Lymphatic channels and that effect play a direct part in lymphoedema.

Pain has no outside or external existence. It is a most personal experience, and cannot be shared by any other person. Pain is an individual experience of ill being. Inadequate management of pain causes impaired function, depression and insomnia. One kind of pain may not be managed by any single medicine or manual practice, because they may have different causes. In Siddha Ayurveda there is no particular uniform medicine for any kind of pain. Management of pain depends upon the causative factor or *Doshik* predominance responsible for the pain. In conventional (allopathic) pain treatment a number of analgesics, anti-inflammatory, anti-pyretic drugs, chemotrypsin and serratiopeptidase like chemicals and opioids are used, but there is no universal drug for all kinds of pain till date. Every individual responds to pain in a different way. In the same way, every analgesic chemical acts pharmacologically in a different way. Only one analgesic preparation cannot solve the problem of pain. So, the management of pain is not so simple and satisfactory with the aforesaid drugs. Instant pain relief is the motive of *Varma* therapy. Stimulation of *Varma* can produce analgesia by secreting a number of prostaglandin inhibitors, endorphins, interferon and other opioid–like substances which are a hundred times more potent than opium. Instant pain relief by *Varma* therapy is possible within no time. Pain management aims at minimizing distress, and feeling of unrest and improving the quality of life. A cardinal point in the management of pain is that it should be holistic and patient-centered in its application. This can be fulfilled in the Siddha Ayurvedic approach only in terms of *Varma chikitsa*.

Meditation, *Pranayama*, yogic practices and especially *Varma chikitsa* are safe and medicine–free options for conscious relaxation of body and mind. The practice of relaxation results in a reduction of skeletal muscle spasms and a drastic reduction of metabolic activity[16]. It gives a chance to make the body's energy flow in a proper way, uninterruptedly, enhancing physical health. *Varma* therapy must be practiced for a few minutes as the commencement of all physical exercises like yogic exercises and western style exercises. With this, every muscle of the body is persuaded to relax. In a nutshell, we can say that *Varma* therapy is the shortcut key to all aforesaid physical exercises, Yoga and *Pranayama.*

*Varma* therapy is the best technique to attain the effects of *Yoga* and *Pranayama*. It is based on the wisdom of ancient Vedic science and has been formulated in terms of the most suitable technique for the present times.

## Conclusion

The most important outcome of the study which, helps in relieving the symptoms and there is no recurrence within the study duration as well as follow-up done every month for more than eighteen months. This enables the patient to resume day to day activities during the treatment. There was good improvement in all signs and symptoms. The patient was able to do daily routine work without any difficulty. Hence this study has a positive outcome and can be recommended as the therapeutic procedure for Lymphoedema. To a great extent, this study authenticates that *Varma chikitisa* has a good result in treating patients with Lymphoedema. The value of *Varma* therapy is well recognized worldwide as it is harmless, cheapest and easiest therapy in the present times,

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# Antimicrobial activity and phytochemical analysis of *Rivea ornate*

**Karunarathna A.*[1], Jeyadevan J.[1] and Thavaranjit A.[2]**


## Abstract

*Rivea ornate* is an important medicinal plant in the family Convolvulceae. Extracts of young leaves, matured leaves, stem and roots of *Rivea ornata* were obtained by extraction method using hexane and methanol. Antifungal, antibacterial assay, antibiotic resistance and preliminary phytochemical analysis were carried out. Antibacterial activity was determined against *Pseudomonas* sp., *Proteus* sp., *Enterococcus* sp., *Staphylococcus* sp. and *E, coli* and antifungal activity was determined against *Mucor* sp., *Rhizopus* sp. and *Aspergillus* sp. by using the standard agar well diffusion method. Antibiotic resistance of tested bacteria was determined by the disc method against bacitracin, gentamycin, amoxycilin and streptomycin. The standard Mancozeb had no effect on *Rhizopus* sp. Only methanol extract of young leaf showed the higher degree of antifungal activity against *Mucor sp*. and *Aspergillus* sp. Methanol extract of stem inhibited the growth of *Rhizopus* sp. predominantly.

Hexane and methanol extracts of stem showed activity only against *Enterococcus* sp. and *E. coli* respectively. Methanol extract of root only exhibited activity against *Enterococcus* sp. Methanol extract of young leaf had the predominant antibacterial activity among the tested different extracts against the bacterial species. This study also revealed that *E.coli* and *Pseudomonas* sp. had the multi resistance ability for antibiotics. Phytochemical analysis of methanolic extract of aerial parts revealed that the presence of alkaloids, saponins, tannins, coumarins and Cardiac Glycosides. The antimicrobial activity of Methanolic extracts of young leaves, and matured leaves of *Rivea ornata* is due to the presence of bioactive compounds.


**Keywords:** *Rivea ornate*, antibacterial, antifungal, Phytochemical analysis

## Introduction

*Rivea ornata* belonging to the family Convolvulaceae is distributed throughout southern part of India[1]. They are erect shrubs or scandent from a woody rootstock. Leaves are orbicular to reniform. The inflorescence 3-10 flowers. Fruits are subglobose, glossy brown and glabrous. Seeds are brown in colour and are embedded in crumbly crust[11]. In Tamil, it is known as '*Machuttai*', in Sinhala called as "*Dumbutu*" and in folklore, it is well known as '*Baravat*' and '*Phaang*'. The leaves are given after parturition. In folklore, it is used topically in hemorrhagic diseases and piles[1]. *R. ornata* Cleanses the blood and strengthens all the organs of the body. It can be used for rheumatism and diseases such as white disease, body cooling, cooling to the eyes and good for hair growth. It contains iron and should be included in meals[2]. The aerial parts possess anti-inflammatory activity[3]. It was cooked with garlic and consumed as a green leafy vegetable to increase haemoglobin level[4]. Since there is no reported work on antimicrobial activity, the present study was carried out to analyse the bioactive compounds qualitatively and to determine the antimicrobial activity of the extracts of Matured leaves, young leaves, stem and root of *Rivea ornate* (Figure 1).

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**Fig.1: Plant of *Rivea ornate***

## Materials and Methods

### *Preparation of plant extracts*

The healthy plant parts were collected from Navaly area in Jaffna, Sri Lanka. The plant material was taxonomically identified and authenticated by the support from the taxonomist of Department of Botany, University of Jaffna, Sri Lanka. They were dried in shade. Completely dried of Matured leaves, young leaves, stem and root were ground into fine powder using an electric blender. The powder was used to get hexane and methanol extractions as described below.

### *Preparation of Hexane extract*

The n-hexane extracts were prepared by soaking 40 g of dried plant parts powder with 200ml n-hexane in a clean, dry-sealed bottle. Then it was time to time manually shacked well for 48 hours and filtered through Whatman filter paper No 1 in a Buchner funnel. The remaining residue was re-extracted two more times. All three n-hexane extracts were combined and the solvent was removed by using Buchi rotary evaporator under reduced pressure. After that the crude extract was then transferred into the glass vial (table 1) and kept in oven at 40 0C to remove the solvent completely.

### *Preparation of Methanolic extract*

The air-dried remaining residue from above was further extracted with 200ml methanol by shaking for 48 hours as above and it was filtered through Whatman filter paper No. 1 in a Buchner funnel. The remaining residue was re-extracted two times more and the corresponding filtrates were pooled. The pooled filtrate of methanol was concentrated on a Buchi rotary evaporator at 40 0C under reduced pressure. The crude extract was then transferred into the glass vial (table 1) and kept in oven at $40^0$C to remove the solvent completely.

**Table 1: Weight of dried crude extracts**

| Plant part | Hexane extract (g) | Methanol extract (g) |
|---|---|---|
| Young leaf | 1.1 | 1.6 |
| Matured leaf | 0.9 | 1.3 |
| Stem | 0.8 | 1.0 |
| Root | 0.8 | 0.9 |

### *Phytochemical Analysis*

Different types of phytochemical constituents of the *Rivea ornata* young and matured leaves extracts were determined by using standard procedures[5, 6, 12]. The colour intensities of each extract and/or the appearance of solids in those extracts during the identification reactions revealed a semi-quantitative evaluation of the presence of various kinds of secondary metabolites. Standard procedures were used to determine Tannins, saponins, flavonoids, steroids, cardiac glycosides, alkaloids and coumarins.

### *Collection of Microbial cultures*

The fungi *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp. and the bacteria *E. coli*, *Enterococcus* sp., *Pseudo-monas aeruginosa*, *Proteus* sp., *Staphylococcus aureus* were obtained from the culture collections of the Department of Botany, University of Jaffna, Sri Lanka. Fungal cultures were maintained in Potato Dextrose Agar (PDA) medium whereas bacterial cultures were maintained in Nutrient Agar (NA)medium.

### *Determination of antibacterial activity*

Antibacterial activity of *Rivea ornata* extracts of stem, root, young leaves and matured leaves was determined using agar well diffusion method. Young

bacterial suspension of *Pseudomonas aeruginosa* was standardized with 0.5M Mc Farland standard. 0.1 mL of a particular bacterial suspension was spread on the entire surface of the Mueller Hinton agar plate uniformly with the help of a sterile spreader. 9 mm diameter wells were made by using a sterile Corkborer. Then 150 µL of test solutions (500ppm), standard (0.01M Streptomycin), controls (hexane, methanol) were eluted into each well separately with the help of a sterile micropipette. All the plates were incubated at 37°C for 24 hours and the zone of inhibition around the well was measured. Each experiment was repeated thrice and the mean value was obtained. The above procedure was repeated for *E.coli* , *Enterococcus* sp., *Proteus* sp. and *Staphylococcus aureus* bacterial suspensions.[7,9]

***Determination of antifungal activity***

Fungal spore suspension [x* $10^5$ number of spores/mL] of 0.1 mL was spread separately on the entire surface of the PDA plate with the help of a sterile spreader. 9 mm diameter wells were made by using a sterile Corkborer. Then 150 µL of test solutions (500ppm) of stem, root, young leaves and matured leaves extracts, standard (20mg/100mL Mancozeb- Dithane M-45), control (hexane, methanol) were eluted into each well separately with the help of a sterile micropipette. All the plates were incubated at room temperature for 4-5 days and the zone of inhibition around the well was measured after 72 hours. Each of the experiment was repeated thrice and the mean value was obtained. The above procedure was repeated for each of the fungus.[8,10]

***Determination of antibiotic resistance of tested bacteria***

Multiple antibiotic resistance against Bacitracin, Gentamycin, Amoxicillin and Streptomycin of tested bacteria was determined by the disc method on Mueller Hinton agar medium. Zone of inhibition was determined after 24 hours of incubation.

**Results**

The qualitative tests for the presence of phytochemicals revealed that the methanolic extracts of the aerial parts of the *Rivea ornata* presence of alkaloids, saponins, tannins, coumarins and Cardiac Glycosides, whereas flavonoids and steroids were not detected (Table 2).

**Table 2: Phytochemical constituents of methanolic extract of aerial parts of *Rivea ornate***

| Tests for Phytoconstituents | Methanol extract of young leaves | Methanol extract of Matured leaves |
|---|---|---|
| Test for alkaloids. (Mayer's reagent) | +ve | +ve |
| Test for flavonoids. (lead acetate test) | -ve | -ve |
| Test for saponins. (Froth test) | +ve | +ve |
| Test for Cardiac Glycosides (Test with FeCl3) | +ve | +ve |
| Test for Tannins. (Reaction with fecl3) | +ve | +ve |
| Test for coumarins. (EtOH/ KOH) | +ve | +ve |
| Test for steroids ($AC_2O$ + $C.H_2SO_4$) | -ve | -ve |

-ve- absent, +ve - present

Bacterial growth was observed after 24 hours of incubation period. Antibacterial assay revealed that the standard streptomycin exhibits the highest antibacterial activity rather than the concentration of 500 ppm test solutions of stem, root, young leaves and matured leaves extracts and hexane and methanol as control. Antibacterial activity was high against all bacteria except *E. coli, Proteus* but *Pseudomonas* sp., *Staphylococcus* sp., and *Enterococcus sp*. were predominantly inhibited by test solutions compared with controls (Table 3).

**Table 3: Antibacterial activity of different test samples**

| Name of the bacteria | Average Zone of inhibition in mm After 24 hours of incubation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stem | | Root | | Young Leaves | | Matured Leaves | | Control | | Standard |
| | Hex | Meo | Hex | Meo | Hex | Meo | Hex | Meo | hex | Meo | |
| *Pseudomonas sp.* | - | 9.75± 0.95 | - | - | - | 13.25± 0.21 | - | 10.4± 0.8 | 10.3± 0.15 | 9.75± 0.95 | 21.25± 0.06 |
| *Proteus sp.* | - | - | - | - | - | - | - | - | - | - | 17.75± 0.75 |
| *Enterococcus sp.* | 10.75 ±0.06 | - | - | 10.25 ±0.5 | - | 10.25± 0.5 | - | 10.5± 0.92 | - | - | - |
| *Staphylococcus sp.* | - | 12.5± 0.30 | - | 10.25 ±0.5 | | 12.25± 0.06 | 10.25± 0.5 | 13.5± 0.79 | 10.5± 0.3 | 12.5± 0.08 | 19.75± 0.40 |
| *E-coli* | 9.75± 0.96 | 10.5± 0.57 | - | - | - | 11.00± 0.81 | - | - | 11±0.82 | - | - |

The growth of all fungi was observed after 72 hours of the incubation period. It was observed that the diameter of the zone of inhibition decreased with an increasing incubation period. The standard mancozeb exhibited the highest antifungal activity rather than the concentration of 500 ppm test solutions and controls. Antifungal activity was exhibited against all fungi. But *Aspergillus* sp. was predominantly inhibited by test solutions compared with controls. The degree of inhibition depends on the type of fungi, spore concentration, types of antifungal compounds and their concentrations (Table 4).

**Table 4: Antifungal activity of different test samples**

| Average zone of inhibition (mm) after 72 hours of incubation | | | |
|---|---|---|---|
| **Test solution** | ***Mucor sp.*** | ***Rhizopus sp.*** | ***Aspergillus sp.*** |
| Stem (hexane) | _ | _ | _ |
| Stem (methanol) | 12.5 | 18.5 | 12.5 |
| Root (hexane) | _ | _ | _ |
| Root (methanol) | 12.8 | 12.5 | 12.5 |
| Y.leaf (hexane) | _ | _ | _ |
| Y.leaf (methanol) | 16 | 16 | 19.5 |
| M.leaf (hexane) | _ | _ | _ |
| M.leaf (methanol) | 12.25 | 12.25 | 18.5 |
| Hexane (control) | _ | _ | _ |
| Methanol (control) | _ | _ | _ |
| Mancozab (standard) | 18.5 | _ | 15.5 |

Y. leaf-young leaf: M. leaf-matured leaf

**Table 5: Antibiotic resistance of different bacteria**

| Average zone of inhibition in mm after 24 hours of incubation | | | | |
|---|---|---|---|---|
| | Antibiotics | | | |
| **Bacteria** | **Bacitracin** | **Gentamycin** | **Amoxicillin** | **Streptomycin** |
| *Enterococcus sp.* | 12 | 10.5 | 15.5 | _ |
| *Pseudomonas sp.* | _ | 22.5 | _ | 11.5 |
| *E. coli.* | _ | 18.5 | _ | _ |
| *Staphylococcus sp.* | 14.5 | 23.5 | 13.5 | 14.5 |

## Discussion

The present study is to find out the degree of antifungal activity of different extracts of *Rivea ornata* against the tested fungi *Aspergillus* sp., *Mucor* sp. and *Rhizopus* sp. Using fungal conidia or spores as inoculum is more preferred than the fungal hyphae. Because the hyphal form cannot be accurately counted, diluted or transferred because of the adherence of hyphae to the surface and the macroscopic, interconnected nature of the hyphal mat. Due to this reason spores/ conidial suspension was used as the inoculum and these can be easily counted, diluted and transferred. The antifungal activity was detected by the inhibition in the growth of fungal hyphae.

In this study, a synthetic fungicide Dithane M-45 was used as a standard in order to assess the degree of effectiveness of the crude extracts by comparison. The trade name of this fungicide is Mancozeb. This fungicide was used at its recommended dosage (2g/l). Most commonly this can be used to control the diseases like downy mildews, rust diseases, anthracnose and leaf blights in the field crops except rice plants.

The growth of all fungi was observed after 72 hours of the incubation period. The standard mancozeb exhibited the highest antifungal activity except had no effect on *Rhizopus* sp. Methanol, Hexane and all stem, root and leaf hexane extracts did not show any effect on the growth of all tested fungi. Methanol extract of young leaf showed a higher degree of antifungal activity against *Mucor sp*. And *Aspergillus* sp. whereas methanol extract of stem inhibits the growth of *Rhizopus* sp. predominantly.

In the preparation of bacterial suspensions, the turbidity of each suspension was compared with the McFarland standard. Generally, McFarland Standards are used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. A McFarland Standard is a chemical solution of barium chloride and sulfuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulfate. In this study concentration of 0.5 mol $L^{-1}$ McFarland standard was used to compare the turbidity.

In this study an antibiotic Streptomycin was used as a standard in order to assess the degree of effectiveness of the crude extracts by comparison. Streptomycin is an aminoglycoside antibiotic that is produced by the soil actinomycete *Streptomyces griseus*. Normally, it performs by binding to the 30S (Svedberg unit) ribosomal subunit of susceptible organisms and disrupting the initiation and elongation steps in protein synthesis. A concentration of 500 ppm streptomycin solution was used in this study.

The degree of antibacterial activity varies not only with bacterial species but also with the tested samples. The standard streptomycin exhibited the highest degree of antibacterial activity. Hexane and methanol extracts of stem showed antibacterial activity only against *Enterococcus* sp. and *E.coli*

respectively when compared with the control. Methnol extract of root only exhibited activity against *Enterococcus* sp. Young and mature leaf extracts of methanol had activity against four and three bacterial species respectively out of five bacterial species that were tested. Methanol extract of young leaf had the predominant antibacterial activity compared to the tested different extracts against the bacterial species. The degree of antimicrobial activity varies with the species, type of extracts, types of bioactive compounds, growth media and incubation conditions. The phytochemical analysis of the methanolic extract of aerial parts revealed the presence of alkaloids, saponins, tannins, coumarins and Cardiac Glycosides, whereas flavonoids and steroids were not detected[7,8].

*Staphylococcus* sp. was sensitive to all tested antibiotics. *Enterococcus* sp. showed resistance against only streptomycin. *Pseudomonas* sp. exhibited resistance against bacitracin and amoxicillin. But *E. coli* was only sensitive to gentamycin. This study revealed that *E. coli* and *Pseudomonas* sp. had the multi-resistance ability for the tested antibiotics[7, 9].

## Conclusion

The phytochemical analysis of methanolic extract of aerial parts revealed that the presence of alkaloids, saponins, tannins, coumarins and Cardiac Glycosides. Bioactive compounds play a major role in antimicrobial activities. The standard mancozeb had no effect on *Rhizopus* sp. Only the methanol extract of the young leaf showed the higher degree of antifungal activity against *Mucor sp*. and *Aspergillus* sp. whereas the methanol extract of the stem inhibits the growth of *Rhizopus* sp. predominantly. Hexane and methanol extracts of stem showed antibacterial activity only against *Enterococcus* sp. and *E.coli* respectively. Methanol extract of root only exhibited activity against *Enterococcus* sp. Methanol extract of the young leaf had the predominant antibacterial activity among the tested different extracts against the bacterial species. The degree of antimicrobial activity varied with the species, type of extracts, groups of phytochemicals and amount of bioactive compounds, growth media and condition of incubation.

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# Protective activity of *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. and *Withania somnifera* (L.) Dunal against lipid peroxidation, protein oxidation and deoxyribose oxidation

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**Abstract**

*Withania somnifera* L. (family - Solanaceae) and *Tinospora cordifolia* (family - Menispermaceae), medicinal herbs have different biological properties such as anticancer, immunomodulatory, hypoglycemic, anti-hepatotoxic, anti-inflammatory, gastroprotective, antioxidant, radioprotective effects. The present study was carried out to evaluate the protective effects of aqueous extracts of *T. cordifolia* (TC) and *W. somnifera* (WS) against lipid peroxidation, protein oxidation, and deoxyribose oxidation. The potential of inhibition of lipid peroxidation, protein oxidation, and deoxyribose oxidation by different concentrations of TC and WS aqueous extracts was tested with standard protocols. The $EC_{50}$ values for inhibition of lipid peroxidation of *T. cordifolia* (TC) and *W. somnifera* (WS) were 146.2±1.2 µg/mL and 37.1±1.6 µg/mL, respectively. The $EC_{50}$ value obtained for ascorbic acid as a positive control was 47.1±.1.1 µg/mL. Both extracts of TC ($EC_{50}$ 8.0±1.4 µg/mL) and WS ($EC_{50}$ 7.2±1.1 µg/mL) showed higher protective activities against the inhibition of deoxyribose oxidation compared with the positive control Gallic acid ($EC_{50}$ 8.6±1.0 µg/mL). *W somnifera* ($EC_{50}$ 75.5±1.0 µg/mL) and *T. cordifolia* ($EC_{50}$ 112.4±1.7 µg/mL) showed less potential for inhibition of protein oxidation compared to positive control ascorbic acid, which was $EC_{50}$ 57.0±1.3 µg/mL. The potential for inhibition of protein oxidation of both WS ($EC_{50}$ 75.5±1.0 µg/mL) and TC ($EC_{50}$ 112.4±1.7 µg/mL) was less than the positive control ascorbic acid ($EC_{50}$ 57.0±1.3 µg/mL). *W somnifera* exhibited a more potent protective activity against lipid peroxidation, protein oxidation, and deoxyribose oxidation than TC. TC showed moderate activity compared with positive controls. Hence WS and TC may serve as potential sources of natural antioxidants for pharmaceutical applications.

**Keywords:** *Withania somnifera, Tinospora cordifolia,* inhibition of lipid peroxidation, protein oxidation, deoxyribose oxidation

## Introduction

The reactive oxygen species (ROS) are generated in the human body from exogenous chemicals, physical sources, and endogenous metabolic processes. In addition to non-radical species like hydrogen peroxide ($H_2O_2$), ROS comprise free radicals including superoxide (O2•-), hydroxyl radical (•OH), and peroxyl radical (RO2•)[1,2]. The production of excess uncontrolled ROS steers oxidative stress activating free radicals causing cellular injury and the ageing process. The major target of ROS is cellular components, including lipids, DNA, and proteins in the body[3]. ROS cause catastrophic and irreversible damage to proteins, lipids, and DNA due to their high chemical reactivity.

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Lipids are attacked and oxidised by ROS to produce peroxides and aldehydes. Peroxidation of membrane lipids can inactivate cellular components leading to diseased conditions[4,5]. Proteins are also vulnerable to ROS attacks, which can modify their function through nitrosylation, carbonylation, glutathio nylation and the formation of disulfide bonds[6]. Furthermore, site-specific amino acid modification, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electric charge, and increased susceptibility of proteins to proteolysis happen due to excessive ROS production[7]. In recent years, much attention has been focused on ROS, especially in clinical medicine, due to its cause of many degenerative diseases such as atherosclerosis, ischemia-reperfusion, heart failure, Alzheimer's disease, rheumatic arthritis, cancer, and other immunological disorders[8]. Therefore, developing and utilising more effective antioxidants is a timely requirement.

The bioavailability of antioxidants derived from natural sources is higher. It is therefore preferred that natural antioxidants have more protective activity. In general, major health-beneficial substances are natural antioxidants from medicinal plants. β-carotene and other natural antioxidants are essential for avoiding cancer and numerous cardiovascular problems[3]. It is crucial to comprehend these plants' potential toxicity and health advantages. A wide variety of substances, including phenolic compounds, flavonoids, and carotenoids, are natural antioxidants. Recently, various plant materials' antioxidant capacities have been characterized[9]. In the present study, two medicinal plants (*Withania somnifera* L and *Tinospora cordifolia*) were selected to investigate their antioxidant capacities against lipid peroxidation, protein oxidation, and deoxyribose oxidation.

*Withania somnifera* L (family-Solanaceae), commonly known as Ashwagandha. It is widely distributed in India, the Middle East, and parts of Africa. It is a short, delicate, evergreen shrub that is wild-grown and cultivated for medicinal purposes. For more than 2500 years, it has been used as a home remedy for many diseases. The root of *W. somnifera* has long been thought to have the most significant medicinal potential[10]. The roots of *W. somnifera* are rich with several alkaloids, withanolides, a few flavonoids, and reducing sugars[11]. Bishayi *et al* 2002 reported that there are more active compounds in *W. somnifera*, including withaferin A, sitoindosides VII–X, 5-dehydroxy withanolide-R, withasomniferin-A, 1-oxo-5b, 6b-epoxy-witha-2-ene-27-ethoxy-olide, 2,3-dihydro-withaferin A, 24,25-dihydro-27-desoxy withaferin A, 27-O-b-D-glucopyranosylphysagulin D, physagulin D, withanoside I–VII,27-O-b-D-glucopyranosyl-viscosalactone B, 4,16-dihydroxy-5b, 6b-epoxyphy-sagulin D, viscosalactone B and diacetylwithaferin A[12]. Previous research showed that *W. somnifera* has beneficial effects in treating arthritis, geriatric issues, and stress, as well as anticancer, anti-inflammatory, and anabolic activity[13].

Ayurvedic and folk medicine both extensively use the well-known medicinal herb *Tinospora cordifolia*. It is a large, glabrous, succulent climbing shrub from the Menispermaceae family. It has been demonstrated that this plant's roots, stems, and leaves have various therapeutic uses. There have been reports of various pharmacological characteristics, including immunomodulatory,[14,15] hypoglycemic,[10], anti-hepatotoxic, [11,12], anti-inflammatory,[16] antioxidant[17]. Therefore, in the present study, the protective effects of aqueous extracts of *T. cordifolia* and *W. somnifera* against lipid peroxidation, protein oxidation, and deoxyribose oxidation were evaluated under *in vitro* conditions.

## Materials and Methods

### *Preparation of extracts*

The plants were obtained from "Weda Waththa" (6.801746, 79.977027) located in Maththegoda, Colombo district, Sri Lanka from August to September 2021. The plants were identified by a Senior Lecturer at the Institute of indigenous medicine, University of Colombo. The stem (*T. cordifolia*) and root (*W. somnifera*) of the plants were used for the study. Plant parts were cleaned and subjected to freeze drying to avoid oxidation of endogenous substances. After that, plant parts were

ground to a fine powder, 60 g was extracted with 1920 mL of deionised water, and the volume was reduced to 240 mL under low heat. Extracts were freeze-dried, and samples were stored at -20$^0$ C.

***Inhibition of protein oxidation***

The effect of TC and WS aqueous extracts on protein oxidation was carried out using a modified method of Wang and co-workers 2006[18]. A Fenton-type reaction oxidised bovine serum albumin (BSA). Different concentrations (7.81-250 µg mL$^{-1}$) of TC and WS extracts (0.5 mL) were mixed with a reagent mixture (1.5 mL) containing potassium phosphate buffer (PBS) (20 mM, pH 7.4, 300 µL), BSA (4 mg mL$^{-1}$), $FeSO_4$ (2 mM, 300 µL), $H_2O_2$ (30%, 400 µL) and was incubated for 30 min at 37º C. After that 2,4-Dinitrophenylhydrazine (DNPH) (1.0 mL of 10 mM) in 2 M HCl was added to the mixture to determine the protein carbonyl content of the samples. Then, 1.0 mL of cold trichloroacetic acid (TCA) (10%, w/v) was added followed by 30 min incubation at room temperature for 30 min. Then the mixture was subjected to centrifugation at 3000 rpm for 10 min. The resulting protein pellet was washed with ethanol/ethyl acetate (1:1, v/v, 2.0 mL) and the pellet was resuspended in guanidine hydrochloride (6 M, pH 2.3, 1.0 mL). The absorbances of the samples were read at 370 nm wavelength. L- Ascorbic acid was used as the positive control. The following equation calculated the percentage inhibition of protein oxidation.

% inhibition = $(A_{control} - A_{sample})/A_{control} \times 100$

$EC_{50}$ value was calculated using a standard graph constructed with different concentrations vs % inhibition.

***Inhibition of lipid peroxidation***

The inhibition of lipid peroxidation was evaluated by the method of Dhar et al. 2013[19]. The egg yolk was used as the lipid-rich source to form lipid peroxides. Briefly, 1.15% w/v KCl was added to the fresh egg yolk emulsion to prepare a 10% v/v solution. Then different concentrations (7.81-250 µg/mL) of TC and WS extracts were mixed with egg yolk emulsion solution (50µL), and trichloroacetic acid (20% aqueous, 150 µL) and thiobarbituric acid (150 µL, 0.67% w/v ) added respectively. The reaction mixture was incubated at 95° C in the water bath for 1 hour, followed by the vortex. The mixture was subjected to centrifugation at 3000 rpm for 10 min. The absorbance of the upper layer was measured at 532 nm wavelength, and percentage inhibition was calculated with the following formula.

% Inhibition = $(A_{control} - A_{sample})/A_{control} \times 100$

$EC_{50}$ value was calculated using a graph constructed with different concentrations vs % inhibition. Results were compared with positive control L- Ascorbic acid

***Inhibition of deoxyribose oxidation***

The inhibition of deoxyribose oxidation was measured according to the modified method of Halliwell 1987[20]. The absorbance was obtained at 532 nm and compared with the positive control, Gallic acid. The percentage inhibition of deoxyribose oxidation was calculated with the following formula.

% inhibition= $(A_{control} - A_{sample})/A_{control} \times 100$

$EC_{50}$ value was calculated using a graph constructed with different concentrations vs % inhibition.

**Statistical analysis**

All the results were expressed as the mean ± standard deviation (Mean ± SD) of at least three independent experiments. Calibration curves were considered linear if $R^2 > 0.99$. The $EC_{50}$ values were calculated from linear dose-response curves where $R^2 > 0.95$. The paired t-test was used for the statistical analysis, and all analyses were done using graph pad prism (2010) statistical software.

**Results**

Inhibition of lipid peroxidation by aqueous extracts of *Tinospora cordifolia* and *Withania somnifera* and standard (Ascorbic acid) is shown in Figure 1.

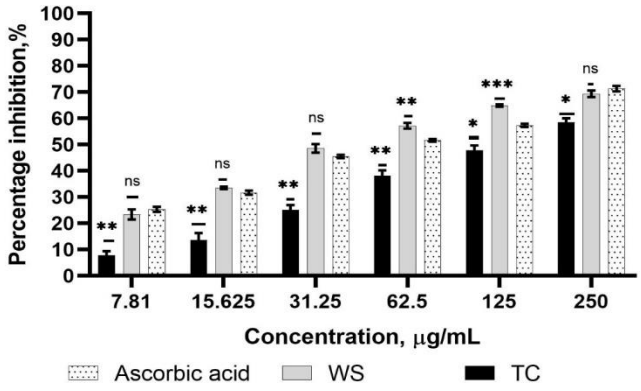


**Figure 01**: **Inhibition of lipid peroxidation by aqueous extracts of *Tinospora cordifolia* and *Withania somnifera* and standard (Ascorbic acid)**
P values are represented as *< .05, ** < .01 and *** < .001 in comparison to the control (Ascorbic acid).

The aqueous extracts of WS showed significant inhibition of lipid peroxidation compared with the positive control ascorbic acid ($p < .001$) (Figure 01) and, WS exhibited a 50 % inhibition of lipid peroxidation at 37.1±1.6 µg/mL. The TC exerted moderate protective activity against lipid peroxidation compared with ascorbic acid. The EC50 value for the TC was recorded as 146.2±1.2 µg/mL (Table 01).

**Table 01: $EC_{50}$ values of inhibition of lipid peroxidation by *Tinospora cordifolia*, *Withania somnifera* and Ascorbic acid**

| Positive control/Plant extracts | $EC_{50}$, µg/mL |
|---|---|
| *T. cordifolia* | 146.2±1.2 (***) |
| *W. somnifera* | 37.1±1.6 (***) |
| Ascorbic acid | 47.1±.1.1 |

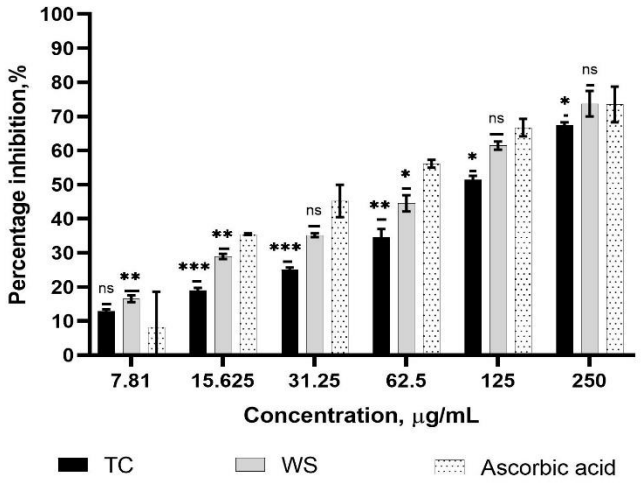


**Figure 02: Inhibition of protein oxidation by aqueous extracts of *Tinospora cordifolia* and *Withania somnifera* and standard (Ascorbic acid)**
P values are represented as *< .05, **< .01 and *** < .001 in comparison to the control (Ascorbic acid).

The effect of aqueous extracts of TC, WS, and ascorbic acid against protein oxidation are shown in Figure 2. TC and WS exhibited a dose-dependent reduction of albumin oxidation, induced by the $H_2O_2/Fe^{3+}$system, which resulted in the formation of a carbonyl group. Fifty percent of protein oxidation inhibit by TC and WS at 112.4±1.7 µg/mL and 75.5±1.0 µg/mL, respectively (Table 2). The effect of ascorbic acid at 57.0±1.3 µg/mL concentration exhibited 50% inhibition.

**Table 02: $EC_{50}$ values of inhibition of protein oxidation by *Tinospora cordifolia*, *Withania somnifera* and Ascorbic acid**

| Positive control/Plant extracts | $EC_{50}$, µg/mL |
|---|---|
| *T. cordifolia* | 112.4±1.7 (***) |
| *W. somnifera* | 75.5±1.0 (***) |
| Ascorbic acid | 57.0±1.3 |

Figure 3 shows the Inhibition of deoxyribose oxidation by an aqueous extract of TC and WS and standard (Ascorbic acid). TC and WS inhibit the oxidation of deoxyribose in a dose-dependent manner. Interestingly WS and TS exhibit potent protective activity compare with the standard. The $EC_{50}$ value of TC and WS is 8.0±1.4 µg/mL and 7.1±1.1 µg/mL, respectively (Table 03).

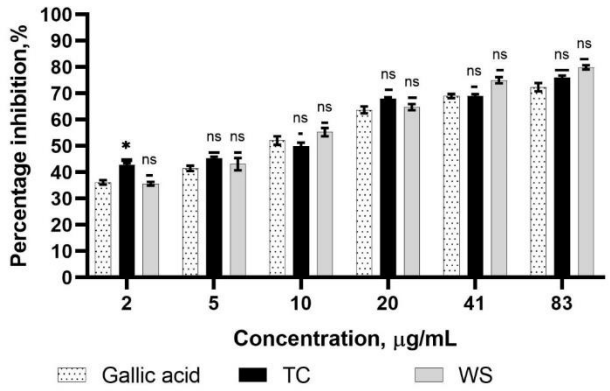


**Figure 03: Inhibition of deoxyribose oxidation by aqueous extract of *Tinospora cordifolia, Withania somnifera* and standard (Ascorbic acid).**

P values are represented as *< .05, **< .01 and *** < .001 in comparison to the control (Ascorbic acid).

**Table 03: $EC_{50}$ values of Inhibition of deoxyribose oxidation by *Tinospora cordifolia, Withania somnifera* and Ascorbic acid.**

| Positive control/Plant extracts | $EC_{50}$, µg/mL |
|---|---|
| *T. cordifolia* | 8.0±1.4 (**) |
| *W. somnifera* | 7.1±1.1 (**) |
| Ascorbic acid | 8.6±1.0 |

## Discussion

Antioxidants are synthetic or natural chemical substances capable of reducing or preventing cellular damage. Utilising natural antioxidants can reduce oxidative damage via direct scavenging of intra- or extra-cellular reactive molecules and activation of cellular antioxidant mechanisms leading to prevent diseases with minimum side effects. The Most natural non-enzymatic antioxidants from natural sources such as diet, plants, fungus, other microbes, animals, etc. Plants are the primary source of dietary antioxidants. Natural products are used directly as medication in traditional and Ayurveda medicine. One of the major bioactivities of antioxidants are the inhibition of lipid peroxidation, protein oxidation and deoxyribose oxidation. The present study evaluated the protective activities of aqueous extracts of TC and WS against lipid peroxidation, protein oxidation, and deoxyribose oxidation.

Lipid peroxidation is the reaction between unsaturated lipids and reactive oxygen species[21]. Malondialdehyde (MDA) is one of the final products of polyunsaturated acids peroxidation form in the cells[22]. MDA level is commonly recognised as a marker of oxidative stress and overproduction of MDA due to increased free radicals[23]. Thiobarbituric acid (TBA) is a common method used to determine the degree of malondialdehyde (MDA) compound in a biological solution [24].

The mechanism of the assay is that MDA reacts with TBA and produces a pink colour which reads at 532 nm. A higher reduction of lipid peroxidation is observed in WS extract ($EC_{50}$, 37.1±1.6 µg/mL) in comparison to the positive control (Ascorbic acid $EC_{50}$ 47.1±.1.1 µg/mL) (Table 01). Molecules present in the WS extract may have a higher contribution to the inhibition of lipid peroxides. The results of the present study suggest that plant extracts can reduce cell membrane damage by scavenging lipid peroxides. IC50 values for the extract and standard trolox were 284.13±146.66 g/ml and 13.52±0.33 g/ml, respectively, in the earlier study by Chaudhuri et al. (2012)[25], which demonstrated that methanol-water extract exhibited effective suppression of lipid peroxidation. According to Gupta et al. 2003[26], Ashwagandha (*Withania somnifera*) had a concentration-dependent rise in the inhibitory ratio on lecithin peroxidation that reached as high as 77.2± 4.4% at a concentration of 45 g/ml (p 0.05).

Direct oxidant damage to a protein's backbone results in fragmentation and conformational changes in the protein's secondary and tertiary structures. For oxidised proteins, the most frequent harm is the

formation of carbonyls[27]. Protein oxidation levels in the food system may now be measured easily and often using the DNPH derivation approach[28]. In this procedure, DNPH combines with protein carbonyl groups to produce hydrazones, and the absorbance is measured at a wavelength of 370 nm[29].

In the present study, inhibition of protein oxidation by WS and TC was tested. The results revealed that WS contains a moderate potential to prevent protein oxidation ($EC_{50}$, 75.5±1.0 µg/mL, Table 02). The positive control (Ascorbic acid) was $EC_{50}$ 51.2±0.1 µg/mL. The *W. somnifera* extract demonstrated more than 50% suppression of protein oxidation at 10 g/mL in a prior study by Gupta et al. 2003[26]. Therefore, WS is highly applicable for a disease that arises due to increased levels of protein carbonyls, such as neurodegenerative diseases (amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, and Huntington's diseases), cataractogenesis, systemic amyloidosis, muscular dystrophy, progeria, Werner's syndrome, rheumatoid arthritis, and respiratory distress syndrome[30].

DNA damage is one of the major effects of ROS[31]. DNA is the cell's genetic material, and OH- radicals react with all purine and pyrimidine bases. The deoxyribose backbone changes the encoded proteins, which may lead to malfunctions or complete inactivation of the encoded proteins. Further, changes in the nucleotides of one strand can result in mismatches with the nucleotides in the other strand, yielding subsequent mutations[32]. Accordingly, the inhibition of the DNA oxidation power of WS and TC was evaluated in the present study. The hydroxyl radical resulted from the interaction of iron (III)-EDTA and $H_2O_2$ with the ascorbic acid present. Thiobarbituric acid is heated with the attacked pentose sugar 2-deoxyribose at a low pH, producing a pink chromogen whose absorbance can be measured at 532 nm wavelength[33]. Interestingly higher inhibition of deoxyribose oxidation was observed in Both plant extracts (The EC50 value of TC and WS is 8.0±1.4 µg/mL and 7.1±1.1 µg/mL, respectively (Table 03)) when compared with the positive control (Ascorbic acid, $EC_{50}$, 8.7±0.6 µg/mL).

## Conclusion

*Tinospora cordifolia* (TC) and *Withania somnifera* (WS) extracts exhibit a good, conferred protection against biomolecule oxidative damage. Therefore, TC and WS extracts could be a promising antioxidant source for the prevention and/or treatment of oxidative stress-related diseases as it could retard oxidative degradation of protein, lipids, and deoxyribose.

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# In-silico and *in vitro* evidence of anti-dengue viral action in selected Sri Lankan medicinal plants; a narrative review

**Gunasekera K.M.**

## Abstract

More than half a century following the first isolation of the dengue virus, there is yet no effective antiviral agent for the treatment of dengue. For centuries, medicinal plants have been used by traditional medical practitioners for treating all types of infections. Plants are the direct or indirect sources of most approved drugs and synthetic drugs have been modelled on natural products. Screening of phytochemicals in vitro and/or by structure based computational studies are two approaches used in the search of a suitable antiviral agent for dengue. High throughput virtual screening by molecular docking allows for rapid and cost-effective screening of a larger number of compounds. This is faster and cheaper than using laborious in vitro assays for screening. Plant compounds identified by in silico assays, could subsequently be confirmed by in vitro assays. In this review, 52 medicinal plants used in Sri Lankan traditional medicine for fever patients, were identified. Literature search in PubMed and SCOPUS databases identified eight of 52 plants (*Acorus calamus, Aegle marmelos, Azadirachta indica, Carica papaya, Glycyrrhiza glabra, Psidium guajava, Syzygium aromaticum* and *Vetiveria zizanioides*) that had been studied by in silico methods. Phytochemicals of these eight plants with good docking activity for dengue virus, are described in this review. Out of these, phytochemicals epicatechin, kaempferol-3-o-β-rutinoside, rutin, catechin, quercetin, chalcones, hesperidin and naringin are the only compounds that have been studied by both in silico and cell culture methods. Except for rutin and hesperidin, in silico findings of all the other compounds were compatible with results of cell culture assays. Phytochemicals with good docking activity for dengue virus target proteins, but which require evaluation by cell culture assays, have been highlighted for consideration in future studies.

**Keywords**: Dengue, Sri Lanka, Plants, In silico, *In vitro*, Phytochemicals

## Introduction

Dengue is a re-emerging infection mainly in tropical and subtropical regions of the world and a major public health problem.[1] Dengue virus (DV) belongs to the genus Flavivirus of the family Flaviviridae. It is an enveloped, single stranded positive sense RNA virus of approximately 11kb genome size. Dengue infection caused by any of the four serotypes (DV1, DV2, DV3 or DV4), may be followed by asymptomatic infection, dengue fever or severe dengue with haemorrhage and shock.[2] Majority of the evidence suggests that high virus loads lead to severe dengue infections.[2] Therefore, it follows that early treatment with an effective antiviral agent, would lead to lower viral loads and less of severe dengue cases.

The dengue virus genome codes for three structural proteins (capsid, membrane precursor, and envelope) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5).[2] Envelope (E) protein plays a vital role in the entry of the virus into host cells making it a major target for drug development.[2] NS3 has a protease and helicase domain. NS2B acts as a cofactor for NS3.[2] NS2B-NS3 serine protease performs the vital function of cleavage of viral polyprotein at the cleavage sites NS2A/NS2B, NS2B/NS3, NS3/NS4A, NS4B/NS5 and at the viral capsid.[2] NS5 protein has a

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methyltransferase domain at its N-terminal end and a RNA-dependent RNA polymerase (RdRP) at its C-terminal end.[2]

Research into dengue started in year 1943-44 when the virus was first isolated.[3] More than half a century later, there is yet no effective antiviral agent for the treatment of dengue. Several approaches have been used in the search for dengue antivirals: 1) repurposing of existing pharmaceuticals, 2) screening of compounds in vitro and 3) structure based computational studies.[4] A number of clinical trials with repurposed pharmaceutical agents, such as chloroquine,[5] prednisolone,[6] balapiravir,[7] celgosivir,[8] ribavirin,[9] and lovastatin[10] have had disappointing outcomes. Other investigators have selected medicinal plants used in traditional medicine, to search for antiviral agents[11].

There is a vast reservoir of lead compounds in nature that could be used either directly or serve as lead structures for the development of new anti-dengue viral agents.[4] Plants are the direct or indirect sources of approximately 50% of approved drugs and seven out of ten synthetic drugs are modelled on a natural product.[12] Medicinal plants have been used by traditional medical practitioners for treating infections for centuries. In Sri Lankan traditional medicine local plants have been used as remedies in fever patients.

Recent computational advances have opened up a new platform for drug development.[13] High throughput screening methods such as in silico experiments can identify substances specific for target sites on pathogens. Several steps in the dengue virus replication cycle have been targeted by in silico studies. The dengue envelope, non-structural proteins NS2B-NS3 and NS5 are the most common putative drug targets selected in studies. Recently a highly potent virus inhibitor, JNJ-AO7, which blocks the interaction between NS3 and NS4B viral proteins was reported.[14] Numerous pharmaceutical agents, phytochemicals and chemicals that have good docking activity with target sites on dengue virus have been reported.[13] These compounds could be used as natural leads or synthetic analogues and their derivatives to produce effective anti-dengue viral agents. However, only a few of these identified compounds have been further evaluated by cell culture or in vivo experiments. High throughput virtual screening by molecular docking allows for rapid and cost-effective screening of a large number of compounds, unlike the more expensive and laborious method of cell culture.

The objective of this review was to identify medicinal plants used in Sri Lankan traditional medicine for treating fever patients, and to describe those that have been studied by computational methods and found to have phytochemicals with good docking activity for dengue virus targets. Phytochemicals that require further confirmation by in vitro and/or in vivo studies have also been highlighted.

Fifty-two medicinal plants, used for treating fevers in Sri Lankan traditional medicine, were identified by consultation with traditional medical practitioners. A literature search for the 52 plants was done in PubMed and SCOPUS databases using the keywords “dengue AND (*name of plant*)”. Criteria for inclusion were: publications prior to the first June, 2020, English language original articles, plant derived bioactive compounds only and in silico studies of phytochemicals that bind to dengue virus targets. Review articles were excluded. A secondary search for additional articles was done manually by scrutinizing references of chosen articles.

The phytochemicals identified as having potential medicinal value were searched on PubMed for in vitro or in vivo studies using “(name of phytochemical) AND dengue” as the keywords. Phytochemicals that had undergone testing in cell culture were identified. Only studies where virus inhibition was measured by plaque reduction assays were included as that is considered the gold standard test for infectivity. An antiviral agent of clinical value should be capable of reducing the dengue virus titre by at least one log (90-100%).

The 52 medicinal plants used in Sri Lankan traditional medicine to treat fever patients were: *Acorus calamus, Aegle marmelos, Aerva lanata, Alastonia scholaris, Alysicarpus vaginalis,*

*Andrographis paniculata, Azadirachta indica, Carica papaya, Carissa carandas, Cassia auriculata, Cassia fistula, Cedrus deodara, Cissampelos pareira, Coriandrum sativum, Coscinium fenestratum, Cuminum cyminum, Curcuma longa, Cyperus rotundus, Foeniculum vulgare, Glycyrrhiza glabra, Gmelina arborea, Gymnema sylvestre, Justicia adathoda, Mollugo cerviana, Munronia pinnata, Myristica fragrans, Oroxylum indicum, Phyllanthus emblica, Piper longum, Piper nigrum, Plumbago indica, Pongamia pinnata, Psidium guajava, Punica granatum, Saussurea lappa, Solanum melongena, Solanum xanthocarpum, Stereospermum suaveolens, Strychnos potatorum, Syzygium aromaticum, Tephrosia purpurea, Tephrosia tinctoria, Terminalia bellirica, Terminalia chebula, Tinospora cordifolia, Tragia involucrata, Tribulus terrestris, Trichosanthes cucumerina, Vetiveria zizanioides, Vitis vinifera, Withaniya somnifera* and *Zingiber officinale.*

An initial search in PubMed and SCOPUS databases using "dengue AND (*plant name*)" as keywords turned up 139 and 254 articles respectively (Figure 1).

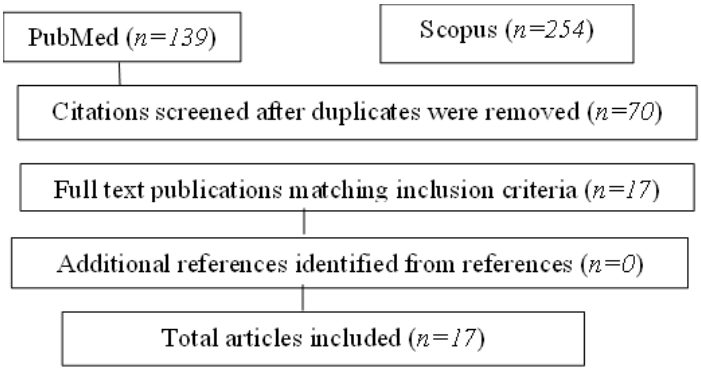


**Fig:1: PubMed and SCOPUS databases using "dengue AND (plant name)**

Only eight of the 52 plants had been screened by in silico methods for dengue virus (Table 1). Phytochemicals of these eight plants, identified as potential natural leads by docking studies, are listed in Table 2. These compounds were searched on PubMed to identify those that had also been studied *in vitro* and/or *in vivo* (highlighted in Table 2). Findings of these studies are described below.

**Table 1. Medicinal plants used for treating fever patients in Sri Lankan traditional medicine and have been studied by in silico methods for dengue antiviral activity**

| Scientific name | Local name | Common English name | Used part of plant | References |
|---|---|---|---|---|
| *Acorus calamus* | Wada kaha | Sweet Flag, Calamus | Rhizomes | 15 |
| *Aegle marmelos* | Beli | Stone apple | Leaves | 16 |
| *Azadirachta indica* | Kohomba | Neem, Margosa | Leaves, seeds, roots, bark | 16, 17, 18, 19, 20 |
| *Carica papaya* | Papol | Papaya | Leaves | 21, 22, 23, 24, 25, 26 |
| *Glycyrrhiza glabra* | Welmee | Licorice | Root | 27 |
| *Psidium guajava* | Pera | Common guava | Leaves | 28 |
| *Syzygium aromaticum* | Karabu-neti | Cloves | Flower, buds | 29 |
| *Vetiveria zizanioides* | Seven-dara | Vetiver | Roots | 30 |

Plant compounds identified with anti-dengue viral activity by in-silico methods and cell culture assays are shown in Table 2.

**Table 2. Plant compounds identified with anti-dengue viral activity by in-silico methods and cell culture assays**

| No. | Scientific name | In silico target site | Compounds identified by in-silico studies | In silico binding | | In vitro activity | |
|---|---|---|---|---|---|---|---|
| | | | | +/- | Ref | Y/ND | Ref |
| **1** | *Acorus calamus* | NS5 | Acoric acid 3 | + | 15 | ND | |
| | | | B-asarone | + | | ND | |
| | | | Calamusin D | + | | ND | |
| **2** | *Aegle marmelos* | NS2B-NS3 | Marmesinin | + | 16 | ND | |
| **3** | *Azadirachta indica* | NS2B-NS3 | Desacetylnimbin | + | 18 | ND | |
| | | | Desacetylsalannin | + | 18 | ND | |
| | | | **Epicatechin**[#] | + | 17 | Y | 17 |
| | | | Hyperoside | + | 17 | ND | |
| | | | **Kaempferol-3-O-β-rutinoside**[#] | + | 17 | Y | 17 |
| | | | Nimbin | + | 18 | ND | |
| | | | **Rutin**[#] | + | 17 | Y | 32, 33, 34 |
| | | NS3 | Meldenin | + | 16 | ND | |
| | | E | Nimbin | + | 19 | ND | |
| **4** | *Carica papaya* | RdRP | Cardenolide | + | 26 | ND | |
| | | | Caricaxanthin | + | 26 | ND | |
| | | | Carpaine | + | 26 | ND | |
| | | NS2B | **Catechin**[#] | + | 22 | Y | 28 |
| | | NS3 | | | | | |
| | | NS5 | | | | | |
| | | NS2B-NS3 | Caffeic acid | - | 21 | ND | |
| | | NS1 | | + | 24 | | |
| | | NS2B-NS3 | Chlorogenic acid | - | 21 | ND | |
| | | RdRP | | - | 26 | | |
| | | NS1 | | + | 24 | | |
| | | E & NS1 | Chymopain | + | 23 | ND | |
| | | NS2B-NS3 & NS1 | Crotonoyl bromide | + | 31 | ND | |
| | | RdRP | Dehydrocarpaine I and II | + | 26 | ND | |
| | | NS1 | 5,7 dimethoxycoumarin | + | 24 | ND | |
| | | NS2B | Epigallocatechin | + | 22 | ND | |
| | | NS3 | | | | | |
| | | NS5 | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2B-NS3 | | - | 21 | Y | 17, 35 |
| | | E & NS1 | **Kaempferol**[#] | + | 23 | | |
| | | NS1 | | | 24 | | |
| | | NS2B, NS3, NS5 | Protocatechuic acid | + | 22 | ND | |
| | | NS1 | | | 24 | | |
| | | NS2B-NS3 | | - | 21 | | |
| | | NS2B-NS3 | | + | 21 | Y | 28, 36 |
| | | NS1 | **Quercetin**[#] | | 24 | | 34 |
| | | E | | | 25 | | |
| | | RdRP | Violaxanthin | + | 26 | ND | |
| | | RdRP | Zeaxanthin | + | 26 | ND | |
| **5** | *Glycyrrhiza glabra* | NS2B-NS3 | 3,3',5'- tetrahydroxy-5-prenylbibenzyl | + | 27 | ND | |
| | | | 3,3',5'-trihydroxy-4-methoxy-5-prenylbibenzyl | + | 27 | ND | |
| | | | 3-acetoxy-4',5-dihydroxy-3'-prenyldihydrostilbene | + | 27 | ND | |
| | | | Licobenzofuran | + | 27 | ND | |
| | | | Glycyrrhisoflavone | + | 27 | ND | |
| | | | 4'-O-methylglycyrrhisoflavone | + | 27 | ND | |
| | | | **Chalcones (kanazol Y)**[#] | + | 27 | Y | 37 |
| | | RdRP + E | **Chalcones (kanazol Y)**[#] | + | 27 | Y | 37 |
| | | methyltransferase | Glabraisoflavanone* | + | 27 | ND | |
| **6** | *Psidium guajava* | E | **Catechin**[#] | - | 28 | Y | 28 |
| | | NS5 | | + | | | |
| | | E | **Hesperidin**[#] | + | | Y | |
| | | NS5 | | + | | | |
| | | E | **Naringin**[#] | + | | Y | |
| | | NS5 | | + | | | |
| | | E | **Quercetin**[#] | - | | Y | |
| | | NS5 | | + | | | |
| **7** | *Syzygium aromaticum* | NS2B-NS3 | Eugeniin | + | *29* | ND | |
| | | | Isobiflorin | + | | | |
| | | | Biflorin | + | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **8** | *Vetiveria zizanioides* | NS2B-NS3 | Ethyl 4-(4-methylphenyl)-4-pentenoate | + | 30 | ND |

#Phytochemicals that have been studied in cell culture
+ good molecular docking
- poor molecular docking
ND – in vitro testing not done
Y – cell culture studies done
E – envelope protein
NS2B-NS3 serine protease
NS5 – non-structural protein 5
NS1 – non-structural protein 1
RdRP – RNA dependent RNA polymerase
Ref – references

***Acorus calamus***

Although in silico findings of this plant have not been confirmed by cell culture methods, good binding of dengue NS5 protein with calamusin D (-6.1 kcal/mol), acoric acid (-5.5 kcal/mol) and β-asarone (-4.7 kcal/mol) have been reported.[15]

***Aegle marmelos***

One computer modelling study was retrieved for marmesinin of *A. marmelos*. Marmesinin had good interactions (-42 kcal/mol) with dengue virus-2 (DV2) NS3.[16] This needs confirmation by cell culture methods.

***Azadirachta indica***

Five studies fitting the inclusion criteria were retrieved for this plant. Forty-nine bioflavonoids from *A. indica* were virtually screened in one study, resulting in the identification of kaempferol-3-O-β-rutinoside (-9.555 kcal/mol), rutin (-9.324 kcal/mol), hyperoside (-7.879 kcal/mol) and epicatechin (-7.622 kcal/mol) as potent dengue virus NS2B-NS3 inhibitors. These bioflavonoids had significant bioavailability and drug-likeness.[17] In-vitro antiviral activity of kaempferol-3-O-β-rutinoside and epicatechin against DV2 showed 77.7% and 66.2% inhibition in baby hamster kidney (BHK-21) cells, substantiating the findings of docking studies.[17] However, the degree of inhibition of dengue virus in vitro by these bioflavonoids was not adequate (<90%).

Three triterpenoids from neem i.e. nimbin (-5.56 kcal/mol), desacetylnimbin (-5.24 kcal/mol) and desacetylsalannin (-3.43 kcal/mol) had a good binding affinity with dengue virus NS2B-NS3 in another study.[18] Nimbin also showed high binding activity against the envelope protein of all four dengue serotypes and had increased absorption and oral bioavailability.[19] These triterpenoids have not been evaluated in cell culture.

Despite promising docking results with NS2B-NS3, the polyphenol rutin did not show significant inhibitory activity with macrophages infected with DV2 and dengue virus–3 (DV3).[17,33] Rutin did not inhibit DV2 replication in African green monkey kidney (Vero) and BHK-21 cells either,[32,34] Meldenin from *A. indica* had good interactions with DV2 NS3 protein but cell culture studies are needed for validation of these results.[16]

***Carica papaya***

Senthivel *et al* investigated seven compounds from *C. papaya* leaves and found that the flavonoid quercetin had the highest binding energy.[21] Farooq and others virtually screened, 900 bioactive phytochemicals of *C. papaya* resulting in the identification of nine compounds i.e. protocatechuric acid, genistein, epigallocatchin, baicalein, 1-hydroxy-2-propanone, catechin, fisetin, 2-methyl-propanoic acid and 2-methyl-butanoic acid, that had high affinity binding to NS2B, NS3 and NS5 proteins of DV2. Epigallocatchin (-13.2911 kcal/mol), catechin (-9.0122 kcal/mol) and protocatechuric acid (-7.5592 kcal/mol) were found to have the highest interaction with NS2B, NS3 and NS5 proteins.[22]

One study screened 103 lead compounds from 43 herbal sources by molecular docking. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one), a natural flavonol present in *C. papaya*, had good binding potential with envelope (-7.2 kcal/mol) and NS1 proteins (-7.5 kcal/mol). Chymopain (disodium;4,5-dihydroxybenzene-1,3-disulfonate) binding energy for envelope and NS1 proteins were -6.5 kcal/mol and -5.9 kcal/mol respectively.[23].

Mir and others identified quercetin (-8.48 Kcal/mol) as the flavonoid with best binding activity to envelope protein by screening baicalein, fisetin, hesperetin, naringenin/naringin and rutin. Baicalein and fisetin although binding well had poor bioavailability. Radakrishnan and others screened nine ligands from *C. papaya* leaves all of which docked well with RdRP. Violaxanthin had the highest interaction energy (-59.17 kcal/mol) and p-coumaric acid showed the least interaction energy. Carpaine, dehydrocarpaine I and zeaxanthin and cardenolide had the potential to dock with DV3 RdRP.[26]

Another study found good binding of six phytochemicals from *C. papaya* against DV2 NS1 protein i.e. kaempferol (-8.1kcal/mol), quercetin (-8.0 kcal/mol), chlorogenic acid (-7.6 kcal/mol), dimethoxycoumarin (-6.2 kcal/mol), caffeic acid (-6.0kcal/mol) and protocatechuic acid (−5.7 kcal/mol).[24] Crotonoyl bromide (-2.9 kcal/mol) of papaya had good affinity with target receptor NS2B-NS3 although it had the lowest inhibition constant value with NS1.[31]

Apart from kaempferol, catechin and quercetin, none of the other compounds of *C. papaya* have been investigated by in vitro studies. The flavonoid kaempferol did not have direct virucidal activity and did not inhibit the virus in the human origin cell line HEK293T/17.35. Surprisingly, when cultured in BHK-21 cells, there was a significant increase in both the level of infection and virus production as a consequence of treatment with kaempferol.[35] These findings were further confirmed by the demonstration of increased expression of envelope protein in DV infected cells treated with kaempferol.[35]

Three studies were retrieved which had investigated the inhibitory activity of quercetin on DV in cell culture assays.[28,34,36] Zandi and others studied the antiviral activity of four bioflavanoids against DV2 in Vero cells. The level of DV2 RNA production in the presence of quercetin was reduced by 67% compared to non-treated infected cells.[36] Quercetin inhibition post-treatment (quercetin added after inoculating virus) was more significant than in pre-treatment (quercetin added before virus inoculation) assays with a selectivity index (SI) value of 7.07.[36] Quercetin did not have significant direct virucidal activity.[36]

In silico findings for quercetin and catechin, have been confirmed in other studies as well. Vero cells infected with DV2 and treated with quercetin had the highest SI value (34.3) and catechin induced better viral inhibition when added before (100% inhibition) than after (91.8% inhibition) virus inoculation.[28] Quercetin from *Houttuynia cordata* displayed anti-DV2 activity with a SI of 0.88 in BHK-21 cells.[34]

***Glycyrrhiza glabra***

A virtual screening analysis by Powers and other, revealed that prenylated stilbenoids (3,3',5'-tetrahydroxy-5-prenylbibenzyl, 3,3',5'-trihydroxy-4-methoxy-5-prenylbibenzyl, 3-acetoxy-4',5-dihydroxy-3'- prenyldihydrostilbene, licobenzofuran), isoflavanoids (glycyrrhisoflavone, 4'-O-methylglycyrrhisoflavone) and chalcones (kanazol Y) demonstrated outstanding docking properties with DV target NS2B-NS3. In addition, kanazol Y docked well with RdRP and DV envelope protein while glabraisoflavanone bound well with methyltransferase[27].

Except for chalcones none of the above-mentioned compounds of *G. glabra* have been evaluated in cell culture. Patil and others developed a group of structurally complex thienyl chalcones which were tested with DV2. Cyclopropylquinoline analog IV showed moderate inhibition of DV2 in cell culture.[37]

***Psidium guajava***

Trujillo-Correa and others studied five flavonoids (quercetin, catechin, naringin, gallic acid and hesperidin) from *P. guajava* by in silico methods. Out of the five ligands, only naringin (−8.0 kcal/mol) and hesperidin (−8.2 kcal/mol) had good docking scores with the envelope protein of DV. Except for gallic acid all others had good docking scores with DV NS5 protein.[28]

The same study demonstrated that quercetin had the highest SI value (34.3), when DV2 infected Vero cells were treated with quercetin.[28] Gallic acid, naringin and catechin were considered as highly selective (SI values ≥10) whereas hesperidin was considered non-selective (SI value <2) in this study. Gallic acid significantly inhibited viral activity when added both before and after virus inoculation but naringin inhibited DV only when added after virus inoculation. Catechin induced the best viral inhibition when added before (100% inhibition) or after (91.8% inhibition) virus inoculation.[28]

***Syzygium aromaticum***

Eugeniin (-10.2 kcal/mol), isobiflorin (-6.8 kcal/mol) and biflorin (-7.2 kcal/mol) from *S. aromaticum* underwent docking analysis with NS2B-NS3 of DV3 and eugeniin was identified as the most potent inhibitor while isobiflorin and biflorin showed moderate inhibition against dengue virus.[29] These findings have not been confirmed in vitro studies

***Vetiveria zizanioides***

Docking analysis of active compounds of *V. zizanioides* identified ethyl 4-(4-methylphenyl)-4-pentenoate as having the maximum binding affinity to NS2B-NS3 of all dengue serotypes.[30] In vitro studies were not retrieved for ethyl 4-(4-methylphenyl) -4-pentenoate.

**Discussion**

Computer modeling studies give detailed descriptions regarding the interactions of compounds with the target proteins. Computational approaches can be used to screen a large number of compounds at a time for antiviral action.[16] Narrowing down the likely compounds by in silico methods accelerates the screening process of compounds by cell culture. This review was intended to identify phytochemicals that had good docking activity with DV target proteins in order to facilitate further studies of these compounds.

Only eight plants out of 52 had been subjected to docking analysis in this review of Sri Lankan medicinal plants (*A. calamus, A. marmelos, A. indica, C. papaya, G. glabra, P. guajava, S. aromaticum* and *V. zizanioides).* DV target sites most commonly used in these studies were NS2B-NS3, NS1 and RdRP.

***Virus life cycle***

The virus life cycle consists of multiple steps which include viral entry, replication, viral assembly and release. Viral entry is initiated by the fusion of viral membrane with the host cell membrane, followed by endocytosis and the formation of endosomes. The low pH in the endosomes triggers fusion of viral and cell membranes which leads to the disassembly of the virion and the release of RNA into the cytoplasm. The envelope (E) protein is another target site of the dengue virus that has been utilized for the development of antivirals. The virus life cycle is initiated by binding to receptors on the envelope and internalization by endocytosis. The receptors involved in this process are not fully understood. The proposed host cellular receptors include dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), heparan sulphate receptors, mannose receptors and human C-type lectin like molecules.[41]

Following release of viral genome, the positive strand of RNA is translated into a single strand of polyprotein. This polyprotein is cleaved by viral and cellular proteases into three structural and seven non-structural proteins. Following several rounds of viral transcription viral assembly occurs at the endoplasmic reticulum and is released from the cells.[41]

NS5 is the largest and most conserved non-structural protein of flaviviruses which makes it an ideal target

for antiviral agents that could be used for several related viruses. More than 75% sequence homology is found among all four serotypes of dengue viruses. It is an important antiviral target because its enzymatic activity is crucial for virus replication. NS5 has methyltransferase and RNA-dependent RNA polymerase (RdRp) activities.[41]

NS3 protein is a multifunctional protein which has protease, helicase, nucleoside 5' triphosphatase activities. NS3 protease contains two domains: N-terminal protease which cleaves the viral polyprotein precursor into individual proteins and a C-terminal RNA helicase involved in dengue virus genome replication and viral RNA synthesis. Many compounds studied target NS3 protease domain. NS3 protease requires NS2 as a co-factor for its function.[41] Preventing the processing and release of viral proteins from the polyprotein precursor would inhibit viral genome replication.

NS1 is non-structural protein found in different cellular locations. It is present as the endoplasmic reticulum resident form, membrane anchored form and the secreted form. Intracellular NS1 is involved in early viral replication although its specific function is not well understood.[42] NS1 is also postulated to be involved in the development of severe dengue. Any compound capable of suppressing the activity of NS1 should therefore be capable of reducing the number of severe dengue cases.

***Antiviral activity of phytochemicals***

Phytochemicals listed in Table 2 were searched in PubMed for studies with dengue virus infected cell lines. Epicatechin, kaempferol-3-O-β-rutinoside, rutin, catechin, caffeic acid, quercetin, chalcones, gallic acid, hesperidin and naringin were the only phytochemicals that had been studied for anti-dengue viral activity in cell culture assays. Except for rutin and hesperidin, in silico-positive findings for all other compounds were confirmed by the results of cell culture methods. Phytochemicals that have not been evaluated by in vitro assays have been highlighted in Table 2 for consideration in future studies.

Plants have been the sources of approximately 50% of approved drugs and synthetic drugs are usually modelled on a natural product.[13] Phytochemicals with anti-dengue viral activity are important for the identification of natural leads or for drug development from its analogues. As evidenced by some studies computational methods are not always successful in identifying compounds with inhibitory action.[32,33,34] At the same time some compounds identified as promising have not produced adequate inhibition of viruses in cell lines.[17,35] Despite these drawbacks, in silico screening is useful for accelerating the process of screening numerous compounds in a relatively short time.

Molecular docking identified calamusin D, acoric acid and β-asarone of *A. calamus* as having good free energy of binding with NS5.[15] All three phytochemicals identified are present in *A. calamus* rhizomes which is used in Sri Lankan medicine.[38] Further evaluation of these phytochemicals by in vitro experiments are necessary to confirm their usefulness.

Neem leaves, seeds, roots and bark are used in Sri Lankan traditional medicine. About 135 phytochemicals have been isolated from different parts of neem but only a few have been studied.[18,39] Dwivedi and others reported good inhibitory potential of rutin with NS2B-NS3, but this was not evident in any of the three in vitro studies which used human and mammalian cell lines for growing the DV.[32,33,34]

Fruit, flower, seed, leaf, bark and root of papaya tree are known to possess many biologically active compounds. Aqueous papaya leaf extracts have been used as treatment for dengue fever.[40] Several compounds from *C. papaya* have been shown to have potential inhibitory activity by computational methods. However, only catechin and quercetin have been confirmed as potential inhibitors in cell culture.[28,34,36] Kaempferol although detected by docking methods as a good inhibitor of the envelope and NS1 proteins, did not demonstrate inhibitory activity on HEK293T/17 or BHK-21 cells,[17,35]

Several phytochemicals of *G. glabra* listed in Table 2 were found to have good binding energy with

several target proteins of DV.[27] Derivatives of chalcones have been studied in vitro but *G. glabra* phytochemicals need further evaluation in cell culture.[37]

Despite anecdotal evidence for its medicinal properties, *P. guajava* has not been screened for its antiviral phytochemicals until recently.[28] Trujillo-Correa and others confirmed that catechin (91.8% inhibition), naringin and quercetin (100% inhibition) were good inhibitors of NS5 by docking methods and found similar results with Vero cells and DV2. Hesperedin was the only phytochemical that did not perform well in vitro studies.[28]

Only a few of these computer modelling studies have used target proteins from all four dengue serotypes for analysis.[19] Most investigators have used only the DV2 protein structures. In vitro studies with plant extracts have shown differential activity with the four serotypes. As such, in silico studies based on all four dengue serotypes would lead to more accurate predictions and may explain the occasional contradictory findings of in silico and in vitro studies.

As evident by some of these studies, individual phytochemicals had less inhibitory action than the crude extract of the plant. This demonstrates the importance of synergism between compounds in crude extracts.[34] Soil and climate in different geographical locations affect the chemical composition of plants. Therefore, decoctions prepared from herbal aqueous extracts could vary. This could be overcome by combining compounds that act on different viral target proteins to formulate pharmaceutical formulations that can be regulated.

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# Dissemination of knowledge for health and wellbeing: with special reference to Buddhism and Ayurveda

**Gamage C. K.**

**Abstract**

Accessing and disseminating reliable health information is a key component of health literacy. Health literacy denotes of individuals and communities acquiring, process and understanding the basic health information and services regarding their health and wellbeing. Dissemination of knowledge in Ayurveda system of medicine and Buddhism is of utmost importance for diverse audiences. Ayurveda emphasizes complete positive health and spiritual attainments while Buddhism is a deep philosophy that offers advice for the preservation and the well-being of human beings and even the flora and fauna. This paper aims to explore the close affinity between primary concepts of Buddhism and Ayurveda, to identify the informative similarities that exist between both disciplines, to investigate the positive effects of Ayurveda system on Buddhism and to explore how Buddhism contributed to promoting Ayurveda medicine in Sri Lankan society. A documentary analysis was done using the primary sources of *Vinaya pitakaya*, *Vissuddhimagga* and *Vridhatraya* etc. The study concludes that both disciplines are very closely affiliated and run parallel to each other with similar concerns, principles and philosophies. Accordingly, it is very clear that the Buddha is the foremost religious leader who has analyzed comprehensively the mental diseases of human beings. It further reveals that Buddhism contributed immensely to promoting Ayurveda medicine in Sri Lankan society, especially under the patronage of Sri Lankan kings.

**Keywords:** Dissemination of knowledge, Health and Well-being, Health Literacy, Ayurveda Medicine, Buddhism

## Introduction

Buddhism is one of the greatest philosophies among other philosophies prevalent in India. It is a deep philosophy that offers advice for the preservation and the well-being of human beings, and even the flora and fauna. Ayurveda system of medicine is one of the greatest contributions made by India for the welfare of mankind. It emphasizes complete positive health and spiritual attainments too. It is always a way of life that expresses how to maintain and protect the mental and physical health and achieve longevity. It is quite evident that both Buddhism and Ayurveda have existed in Sri Lanka hitherto without any hindrance. The ultimate goal of Ayurveda is physical health while Buddhism appreciates mental health.

In 1948, World Health Organization has defined the concept of Health as, "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity."[1] The health of a person means the health of the physical body and mind together. Health means balance, harmony and equilibrium in all the physiological activities of the body and mind. Balance in bio-humors, tissues and wastes; good digestive power, positive and happy attitude (*Prasannatma*) in senses, mind and soul, indicates the healthy condition of any human being.[2] According to Buddhism, to fulfill the final goal of worldly (*Laukika*) and spiritual *(Lokoththara*) lives of human beings, hygiene is the major supportive factor. Good health is of great value and also necessary for progress in life. It proves the old saying, 'Health is Wealth' and good health can be considered as an investment. There is a close relationship between the mind and the body. Because of this close psycho-somatic relationship, psychological factors influence

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many physical illnesses and physical factors affect many psychiatric illnesses.

Therefore, the objectives of this study are to explore the close affinity between primary health concepts of Buddhism and Ayurveda, to identify the informative similarities that exist between Buddhism and Ayurveda, to investigate the positive effects of Ayurveda system on Buddhism, and to explore how Buddhism has contributed to promoting Ayurveda medicine in Sri Lankan society.

## Materials and Methods

A documentary analysis was done by using primary sources as well as secondary sources of related literature. *Vinaya pitakaya, Dhamma padaya, Vishuddhi margaya, Mahawanshaya* and major Ayurveda books i.e. Charaka, Susrutha and Ashtanga Hrudaya Samhitha used as primary sources. Books and other publications are written on related topics of Buddhism and Ayurveda also used as secondary sources. Collected data was analyzed in a descriptive method used in the field of social sciences.

### *Review of Related Literature*

Various researchers have been engaged in analyzing the interrelationship between Buddhism and Ayurveda in different countries. Kumar and Bhanupriya[3] stressed that by observing the similarities between Buddhism and Ayurveda, it is very clear that Buddhism has a lot of influence on Ayurveda. The aim of both of these schools is to free a man from his sufferings, physical or psychological. Buddhism and Ayurveda both have similar concerns, principles and philosophies to build a healthy society. Priyadarshana[4] pointed out that in many places of the Pali literature, the Buddha is particularly known by two identical terms, namely *Bhisakko* (doctor) and *Sallkatto* (surgeon), proves the role played by the Buddha not as a physician but as a psychiatrist too. He suggested three kinds of significant strategies to control various mental distortions and problematic disorders faced by humans until realize Nibbana. i.e.*Tadanga pahana, Vikkhamhana pahana* and *Sammuccheda pahana* along with noble eightfold path and *Seela.*

Weerapperuma and Weerasooriya[5] highlighted that though Ayurveda evolved earlier than Buddhism, the contents of Ayurveda were documented in the 4th century B.C. The set of doctrines described in *Sadvataya* has to be practiced for a healthy life. When exploring *Sadvataya* and contents of *Maha mangala, parabhava, Bojjanga, Girimananda, Karaneeya metta, Mettanisansa suttas* together with comparable doctrine to improve physical, mental and spiritual well-being in humans could be identified as the signs of Buddhism in *Sadvataya* which has been described in Ayurveda. According to Panday *et. al*.[6] Buddhism and Ayurveda medicine originated in India and both aim at eliminating suffering. The basic doctrines of *Panchamahabuta, Tridosha,* diseases and treatments, medicinal formulations and surgical procedures were all notable in the practice of Ayurveda medicine and Buddhism.

Bandara[7] has pointed out that during the 6$^{th}$ B.C. in India, a well-developed Ayurveda medical system was prevalent. The Buddha was not a professional doctor. Compared with the advancement of modern science the Buddha's knowledge of the human body is almost equal to that of the modern medical field.

Molligoda[8]identified the close affinity of Buddhism and Ayurveda, evaluation of the cross-fertilization of Indian Hindu traditions and Indian Buddhist traditions through Ayurveda, the basis of surgery, medical compounds as referred to in *Vinaya and Sutta pitakas* and also the basics of prevention of diseases are the sub-themes that have been undertaken in her study. Shekara thero[9], mentioned that "As institutions, Buddhism and Ayurveda are two. Yet the goal is the same. The final goal of Buddhism is mental relaxation (*budu dahame parama nishtawa ciththa vimukthiyayi*). However, Buddhism has not forgotten physical health and Ayurveda has not forgotten mental health."

## Discussion

### *The close affinity between primary health concepts of Buddhism and Ayurveda*

### *The concept of Panchamahabhuta*

All of the basic theories of Ayurveda are derived from the concept of *Panchamahabhuta*. According to this

concept, the human body as well as all matters in the universe are composed of five basic elements. It stands for *Akasha bhuta, Vayu bhuta*, *Teja bhuta, Apo bhuta* and *Pritivibhuta*. Even though all the matter of the universe consists of these five elements they do not exist in equal proportions. The human body is a conglomeration of the five elements and if they become imbalanced the body will suffer disturbance. Charaka Samhitha which is one of the core Ayurveda books has explained in detail the connection between the five sensory organs and the five *Mahabhutas*, "The five *Mahabhutas* are *Akasa, Vayu, Agni, Jala* and *Prithivi*. Their attributes are sound, touch, vision, taste and smell respectively."[10] Vagbhata has mentioned that the physical body (*Bhauthika sarira*) is a result of the combination of *Bhutas* and the body. He explained that there are many substances in the body belonging to each *Bhuta* category which can be understood by their physical properties and functions etc.[11]

According to the Buddhist theory, the universe is comprised of four *Bhutas* (*Sanskara*) and all the elements are subjected to perish and decay. They are *Prithivi, Apo, Tejo* and *Vayu*. The Buddha has accepted only four *Mahabhutas* because they are only perceptible through *Indriyarta's*. Since *Akasha mahabhuta* cannot be perceived in a physical matter form, Buddhists have denounced its presence.[12]

***The concept of four noble truths***

Similarly, the prime feature of Buddhism is four noble truths. What are the four truths; suffering, the cause of suffering, complete cessation of suffering, and the path leading to the cessation of suffering. Similar to these four noble truths in Buddhism, Ayurveda is also found on four bases such as disease, cause of disease, healing of disease and therapeutic healing treatment.

There are four steps of the truth of suffering birth, decay, disease and death. Buddhism helps to overcome birth and death. Similarly, Ayurveda highlights winning over decay and disease. When analyzing these facts it is proved that both these philosophies seem to be of two different paths beginning from the same source to reach the same goal.

***Rejection of both ends and practicing of middle path policy***

There is a mutual relationship between Buddhism and Ayurveda that can be seen even in the first discourse, *Dhamma cakkappavaththana suththa* preached by the Buddha. According to the preaching of the Buddha, two edges i.e. *Kama sukhallikanu yoga* and *Aththakilamathanu Yoga* are the two ends that should be avoided by a person who searches for *Nirvana*. Overfeeding or giving the most sophisticated facilities to life is called *Kama sukhallikanu yoga* and lesser feeding or giving much pain to the body is called *Aththakilamathanu yoga*. The Buddha practiced both of these lifestyles which were popularized and practiced in India at that time. As a prince, who had spent 29 years with a wealth of all luxury things, and after the separation of royal life, had spent 6 years as a hermit with many difficulties. In this manner, the Buddha rejected both ends of lifestyles and confirmed the value of the middle path policy to achieve the success of both lives of this birth and the life after death based on hygiene. Noble eightfold path i.e. right understanding, right thought, right speech etc. are the way to peace of mind, happiness, higher wisdom and good health and it directs the middle path policy.

The Ayurvedic theory also rejects *Athiyoga, Ayoga* and *Mithyayoga. Athiyoga* means excessive usage of foods and behaviors, *Ayoga* expresses the idea of lesser usage of foods and behaviors and *Mithyayoga*, improper usage of foods and behaviors etc. These three are equally harmful and became a root cause of various diseases. Therefore, Buddhism and Ayurveda reject both these ends and highlighted the value of practicing the middle path policy.

***The similarity in the classification of diseases in both disciplines***

The clarification and categorization of diseases in Buddhism are similar to the system of Ayurveda. The imbalance of three *Doshas* is called a disease and the balance status of *Doshas* is called healthiness.

Major Ayurveda classics, have used very comprehensive organizational patterns for the categorization of diseases. Charaka classified diseases mainly into three groups endogenous, exogenous and psychic. "There are three types of diseases; endogenous, exogenous and psychic. Endogenous diseases are caused by the morbid dosas of the body; exogenous by demoniac seizures, poisonous substances, wind, fire or trauma. Psychic ones by the association with the agreeable as well as disagreeable things."[13]

Acharya Susrutha had classified diseases mainly into two types; those curable by surgery and those curable by treatments.[14] Again he categorized diseases into three broad groups viz. Psychosomatic diseases, Traumatic diseases and Natural diseases.[15] Vagbhata categorized diseases into three groups i.e. diseases caused by actions of this life, diseases caused by actions of prior life and diseases caused by actions in both lives in Ashtanga Hardaya Samhitha. [16]

According to Buddhism, diseases are two types i.e. *Sharirikaroga* and *Manasika roga*. *Sharirika* and *Manasika*roga gradually affect the body and the mind, and also *Sharirikaroga* can be developed into *Manasika roga*. There are several diseases treated as exogenous diseases and natural diseases which are included in the *Pali suththa* and *Vinaya pitakaya*. The Buddha who paid due attention to curing mental and physical diseases as well as his knowledge about internal organs of the human body, diseases caused to them and medicine recommended for them is quite evident that this fantastic medical knowledge. The Buddha has mentioned in *Girimananda suththa* 66 physical diseases which preached for *Girimanandathero* and *Sanlekha suththa* of *Majjhima nikaya* are included 44 mental diseases. This knowledge as an anatomist is very much wide. *Girimananda suththa* and *Sanlekha sutta* can be cited as the best examples to reveal this great knowledge about mental and physical diseases.

***The spread and institutionalization of buddhism and how it impacted on evolution and revival of Ayurveda in Sri Lanka***

The establishment of Buddhism as an institution has been very helpful for the development of the system of Ayurveda medicine. Treatment (*Vedakama*) and nursing, (*Hedakama*) the two concepts in Ayurveda are similar to the concept of merit (*Kusal*) in Buddhism.

As the written pieces of evidence in books and inscriptions proved that the patronage given by the Sinhalese kings was a significant factor in the development of the Ayurveda system of medicine in Sri Lanka. The inhabitants of ancient Sri Lanka were devoted followers of Buddhism. Therefore, the kings who performed acts of merit gave the highest priority to the provision of medical facilities to the people. "The Mahavamsa reports that the ancient kings who ensured maximum standards of sanitation for the population and the provision of facilities such as hospitals, medicines and food for the sick were considered meritorious acts of the highest quality".[17]

Most of the kings in the past who ruled the country paid more attention to rendering services through activities such as appointing ministers and chief medical personal (*Mahavedana, Sulu vedana*) and establishing hospitals to treat the sick and improve the medical sector. According to the recorded history of the active participation of ancient rulers which rendered to ensure the health care of the public dates back to the 4th century B.C.

King *Pandukabhaya*, the first king of the Anuradhapura kingdom, built the city according to a very systematic plan like a modern city with health and other urban facilities. History recorded that there was a maternity home known as '*Sivika soththisala'*. However, this fact proved that there was a widespread concept of lying- in- homes and hospitals. The first hospital for public health was constructed during the time of the great king *Pandukabhaya* (453 B.C.). He built a large hospital for the sick *Bhikku*s and a lying –in shelter and hall for those recovering from sickness. During his time great care was given to keep the cities clean and in commendable sanitary conditions.[18]

King *Devanampiyatissa* gained credit for building the first Ayurvedic hospital in the world. The medicinal boat, medicinal stone and other medical equipment found there are testimony to the existence of a well-developed medical system.[19] During the reign of King *Dutugemunu* (161-137 B.C.) 18 hospitals he had built in his kingdom and gave food and medicine regularly to the sick.[20] And also *Vijithapura* battle provides ample proof that there was a veterinary medical system in the country as far back as the reign of King *Dutugemunu.*[21]

King *Buddhadasa* (362-409 A.D.) was well-versed in general medicine, psychology and veterinary medicine and he was also a great surgeon who performed a series of surgical operations. He extended his kindness and medical services not only to humans but also to animals. He was attributed with the authorship of '*Sarartha saṃgrahaya'*. Further, he built halls for the benefit of deaf and dumb persons.[22]King *Kassapa* 1V (895 A.D) built several hospitals in Anuradhapura and Polonnaruwa and also was credited as the first person who built a hospital for patients suffering from communicable diseases. King *Parakramabahu*, the Great (1153 - 1186 A.D.) had taken several special steps to promote the health conditions in the country. He had built a large hospital and given people medicinal food. The reign of King *Parackramabahu* VI, gave an invaluable contribution to developing the field of medicine. Mahavamsa has recorded that during the periods of kings *Aggabodhi* VII, *Silamegha*, *Sena* I, *Sena* II, *Dappula* II, *Dappula* III, *Upatissa* II and king *Udaya* were built hospitals in Anuradhapura, Polonnaruwa and the suburban areas.[23]

***Health preservation practices advocated by the Buddha are similar to the basic principles of Ayurveda.***

When the birth of Prince Siddhartha took place in the 6th B.C., the well-established system of medicine in Indian society was Ayurveda. Ayurveda was one of the sixty-four subjects available to be learned by princes of India and prince Siddhartha had to learn Ayurveda, the only treatment, prevalent then. After the Buddhahood, on most occasions, the Buddha performed as an expert physician. The medicine and treatments recommended by the Buddha to Buddhist monks on various occasions are similar to the basic principles of Ayurveda. They are included in the *Bhesajja kandhaka* in *Maha vaggapali* of *Vinaya pitaka*. The Buddha who paid due attention to curing mental and physical diseases as well as his knowledge about internal organs of the human body, diseases caused to them and medicine recommended for them is quite evident that this fantastic medical knowledge.

***The Buddha and Ayurveda system of medicine***

According to the following verse in *Damma padaya*, the Buddha preached the importance of good health and health is the prime wealth (*Arogya paramalabha*) of one who wishes to succeed in life. They are,

*Arogya paramalabha* (Physical well-being), *Santhu tthi paramandhanao* (Mental well-being), *Vissasa paramagnathei* (Social well-being) and *Nibbhanao paramaosukhan* (Spiritual well-being)[24]

In realizing Buddhist objectives, mental health has to be achieved and when the mind falls sick the body too falls sick. As a physician, he treated the sick and cured those suffering in life. When *Kisagothmi* got upset or mentally depressed when her only child expired and *Patachara* went mad or total mental depression when she lost everyone who loved her and those she loved, the Buddha like a great psychologist cured the diseases of *Sansara* totally with the medicine of *Dhamma*. That is why the *Dhamma* was introduced as "*Dhmmosada*". Leading a healthy life would open the door for health and well-being in the next life after death (Spiritual well-being).

***The Buddha, Bhikkhu society and the concept of hygiene***

The Buddha laid down rules for the hygiene and social well-being of the *Bhikku* society by following *Dasa sil* and *Upasampada sil* and also paid special attention to physical hygiene to those novices who intended to the *Sasana* as priests. Therefore, the Buddha has stressed the importance of good health and pointed out that person sufferings from certain kinds of diseases should not be ordained as *Bhikkus*.

Even when he was attending to such affairs as *Pohoya* and confession novices with deformities were not admitted. Even when accepting such items as *Sivupasa* he emphasized that novices should follow health and hygienic principles. Not only that the Buddha has given instructions to *Bhikku* to maintain his residential quarters, utensils and the temple very clean. According to *Vinaya pitaka*, the Buddha inquired about the health and well-being of those *Bhikkus* who came from far-away places.

It was a general practice for monks to inform the Buddha when they got sick. The Buddha not satisfied with the prescription of medicine alone, often attended to and cared for the sick *Bhikkus*. This emphasizes that curing the sick is equated with caring for the Buddha and thereby the eminent place accorded to such health care and nursing in Buddhism.

The Buddha explained the value of Ayurveda medicine and recommended certain medicine for the sick and the same medicine recommended in Ayurveda. There was Bellattisisa thero suffering from a skin disease which puss oozing out of scratches. This illness was called "*Chullkacchabadhaya*" The Buddha prescribed medicine for this skin disease.[25] For a *Bhikk*u bitten by a snake, the Buddha has endorsed the mixture of feces, urine, hot ash and clay. It was called the "*Mahavikataya*".[26] During the life of the Buddha, a certain *Bhikk*u was supposed to be suffering from a disease caused by the evil spirit. Though he was treated by knowledgeable senior *Bhikkus* the patient was not cured. The patient *Bhikku* went to a place where pigs were slaughtered. There he ate raw pork and the raw blood of the pigs. Therewith his disease was cured. As a cure for any disease caused by evil spirits, the Buddha has endorsed the consumption of raw meat and drinking raw blood.[27] Dinking dissolved feces as a cure for drinking poisons was approved by the Buddha.[28] Because a woman gave a certain Bhikku a lure to entice him. The *Bhikku* suffered a mental aberration called *"Sarananka badhaya*" For this sickness, the Buddha has approved that the mud stuck on a plough be dissolved in water and given to the patient.[29]

***Factors that pay attention to the two disciplines of Buddhism and Ayurveda for good health***

Both disciplines have advocated the value of waking up early, balanced diet and adequate intake of pure water, regular exercises, personal hygiene, the importance of sleep and meditation etc. All these are considered essential for good health.

***Importance of waking up early***

A healthy person should wake up early in the morning before sunrise, during the *Brahma muhurtha*. This is the time when our mind is fresh and the surrounding atmosphere is calm and quiet. The time of *Brahma muhurtha* is the best time for praying to '*Brahma*' or '*God*', for meditation, acquiring supreme knowledge and eternal happiness. Vagbhata has mentioned that "the healthy person should get up during Brahma muhurta, to protect his life."[30] It is advisable in Buddhism and Ayurveda to wake in the morning, during the *Brahma muhurta* to protect health.

***Importance of dental hygiene***

Ayurveda advises that food must be well chewed for its proper digestion. For that our teeth should be strong enough. It is essential to calm and brush the teeth and the mouth should be washed in the morning, after lunch or dinner and after eating anything. The practice of cleaning the tongue and gargling the mouth removes bad breath, improves proper taste and appetite and finally solves respiratory system problems.

Buddhism emphasizes the value of dental hygiene to the *bhikku* society by ordering that the young *Bhikkus* should offer the elders' teeth cleaning sticks and powders etc. Cleaning the teeth affects even eyesight, removes bad smells and improves the taste buds.

***Wholesome diet and intake adequate water***

Ayurveda system of medicine emphasized the value of taking a wholesome diet. It is clearly pointed out the suitable diets according to four seasons i.e. Winter, Spring, Summer and Rainy. The importance of getting wholesome food for the three meals, and drinking sufficient amounts of water are also emphasized in the daily regimen.

The Buddha also emphasizes the value of taking enough food for *Bhikkhus* and avoiding bad foods causes many illnesses. Instructions given to

*Sudaththa* (*Anepindu situthuma*) by the Buddha are a very good example of taking the value of enough food for personal hygiene.

***Regular physical exercises***

*Vayayama* or physical exercises are recommended by Ayurveda medicine. It is the best way to reduce the weight of the body and obesity. It enhances the tolerance of fatigue and ensures good health. Lightness (of the body), ability to do hard work, keen digestion, depletion of (excess) fat, stable and distinct physique, accrue from physical exercises.[31]

The Buddha has emphasized the importance of walking as an exercise, specially a remedy for sloth and drowsiness that arises after delicious foods. Jeevaka, the famous physician who treated not only the Buddha but also the *Bhikkus* had recommended the walking compound getting approval from the Buddha for the benefit of *Bhikkus*. The Buddha very often walked for a *Dhamma* preaching, this is called as '*Aturitha chariaka'*.

***Importance of sleep***

Ayurveda emphasizes the value of getting adequate sleep for a healthy life. It is further emphasized that excessive sleep, as well as inadequate sleep and the incorrect ways of sleep harm for health. Proper sleep ensures good health and long life. This points out the truth that sleep is essential as much as food for the prevention of ill health. Buddhism emphasizes that indulging in excessive sleep causes physical and mental decline. Buddhism emphasizes the need of paying attention to everything done and mindfulness even in going to sleep.

***Meditation and reciting Pirith***

Meditation is a psychosomatic and spiritual discipline. It is widely practiced in both disciplines of Buddhism and Ayurveda. Meditation has a significant role to play in improving mental status[32]. It is a therapy with the potential to heal all diseases and more important for good health. It is a way of controlling thought processes and body functions, allowing one's insight to perceive consciousness. It has been practiced for over 4000 years, in the ancient Upanishads and the Vedas and is an integral part of Ayurveda and is also deeply practiced in Buddhism. Reciting *Paritta* (*Pirith)* is one of the parts of the Buddha's teaching. It has a direct psychological effect and it purifies the mental state of the listeners especially of those who are suffering from physical ailments.

By chanting *Pirith*, the Buddha provided relief and blessings to a society that sought the help of various incarnations to cure various physical and mental diseases caused by inhuman effects. Thus, he provided great relief by chanting *Rathana Sutta* to free *Vishala mahanuwara* from three fears, *Karaneeya meththa sutta* to chase away humors caused by ill – spirits to *Bhikkus* meditating in remote jungle areas, *Bojjanga sutta* to cure various diseases and *Atanatiya sutta* to eradicate fear from in humans.[33]

***Importance of separate usage of utensils***

Use of clean instruments used for maintaining health. It has been ordered that the bowls used by *Bhikkus* should be clean, and the robes worn by them also should be clean accordingly, further in walking through villages and settlements slippers should be worn. Using the same bowl, sleeping in the same bed, using the same bedspread and covering the body continuously with one cloth have been prohibited by the Buddha as harmful to health. The system of Ayurveda medicine has always highlighted the value of the above matters for good health.

***Importance of bathing***

The importance of bathing is emphasized by Ayurveda and as well in Buddhism too. Bathing brings cleanliness, increases life span, removal of weariness, prevents perspiration and removes impurities of the physical body. "*Snana* (bath) improves appetite, sexual vigor, a span of life, valor (enthusiasm) and strength; removes itching, dirt, exhaustion, sweat, stupor, burning sensation and sin." [34]

## Conclusion

The aim of both of these disciplines is the same that is to free a man from his sufferings, which may be physical or psychological. By leading an ideal life, it is possible for the whole society to enjoy a healthy life. In this regard, Buddhism and Ayurveda both have similar concerns, principles and philosophies.

Accordingly, it is very clear that the Buddha is the foremost religious leader who has analyzed comprehensively the mental diseases of human beings. The study proves that both Buddhism and Ayurveda have substantiated that physical and mental treatments have been done on the basis of cause and effect. Scholars are of opinion that Buddhism is a "*Nasthika darshana"* and Ayurveda is an "*Asthika darshana"*. Based on the findings, both Buddhism and Ayurveda are very closely affiliated with each other hygienically and run parallel to each other. The study further reveals that Buddhism contributed immensely to promoting Ayurvedic medicine in Sri Lankan society, especially under the patronage of Sri Lankan kings.

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