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Cover story

Nelli

***Phyllanthus emblica* Linn.**
Family: EUPHORBIACEAE
Vernacular names: Sinhala: *Nelli, Ambula;* **Sanskrit:** *Amalaki, Adhiphala;* **English:** *Emblic myrobalan;* **Tamil:** *Amalagam;* **Hindi:** *Amllka, Amalak*

The plant shown on the cover page is *Phyllanthus emblica* Linn. It is a small or middle-sized tree, about 10 m high, with a crooked trunk and spreading branches; bark thin grey with numerous bosses whence arises the leaf bearing branchlets; leaves simple, alternate; Flowers unisexual, small, greenish-yellow, monoecious, appetalous and axillary; fruit globes, 1.2-1.6cm diameter, fleshy, pale green or yellow, of three sub- dehiscent, two seeded, crustaceous cocci enclosed in a thick fleshy coat; seeds 06 trigonous. Fruits are the most utilized part for medicinal preparations[1].

The pericarp of the fruit is often used in decoctions with other ingredients and externally on boils with cow ghee to promote suppuration. The root, bark and fruit are astringent. The unripe fruit is coolong, laxative and diuretic. Exudation from incisions on the fruit is applied externally on inflammation of the eye. The use of the bark with honey and turmeric is given for gonorrhoea. An infusion of the leaves with fenugreek seed is given for chronic diarrohoea. The fruit is rich in vitamin C. The expressed juice of the fruit along with other ingredients is used to cure hemorrhage, anaemia, colic, acute leprosy, fits, insanity, Jaundice, cough, hiccups, indigestion, dyspepsia, asthma and other diseases[2].

Ethnopharmacological studies indicated that 17 countries use *P. emblica* as indigenous medical remedy[3].

The current pharmacological studies indicated that drupes show anti-oxidant and antiproliferative activities attributed to the phenolic compound in the drupes[4].

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Evidence-based traditional medicine for transforming global health and well-being

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Background

In the current Anthropocene epoch, characterized by intensified, human-induced environmental crises, natural disasters the interconnectedness of human health and the health of the planet has become more evident with the resulting responsibility to promote healthy living conditions[1]. In our interconnected world, health challenges transcend borders, and addressing them necessitates comprehensive solutions that consider the complex interplay of factors influencing health outcomes. The COVID-19 pandemic has shown that current healthcare systems have limitations and vulnerabilities. This highlights the importance of adopting preventive and health-promoting strategies that go beyond national boundaries. Concepts such as planetary health and One Health are emerging as integrated, unifying strategies to optimize the health of people, animals, plants, and the planetary ecosystem.

To address the complex global challenges of the 21st century including: geopolitical conflicts, economic crises, environmental disasters, and pandemics, it is crucial to rethink healthcare. This reimagining is essential for successfully reaching the Sustainable Development Goals (SDGs) and creating a better and more sustainable future for everyone. It is critical to carefully address the social and environmental determinants of health beyond conventional notions of healthcare limited to sick care. is critical. Agreeably, healthcare must prioritize explicitly the well-being and prosperity of individuals and communities looking more broadly at social policies impacting health such as agriculture and food, transportation, city planning, housing, racism, sexism, gun safety, criminal justice, war and peace. Embracing principles such as Universal Health Coverage (UHC), preventive healthcare, multi-sectoral collaborations, social justice, environmental responsibility, and digital technology innovations can pave the way for more equitable and resilient societies.

Governments and agencies worldwide recognize the need for transformative reforms through conducive policies and declarations. The Declaration of Astana 2018, the Helsinki Declaration 2020, and the Geneva Charter for Well-being 2021, developed by the World Health Organization (WHO), advocate a well-being economy with a primary focus on primary healthcare to achieve equity, social justice, and community empowerment. Moreover, the Shanghai Declaration 2016 and WHO Traditional Medicine Strategy acknowledge the growing importance of Indigenous Knowledge (IK) and Traditional Medicine (TM) in advancing health goals. There is a growing consensus to decolonize the restrictive idea of IK/TM and expand its vision to include traditional, complementary, and integrative medicine and health systems.

For centuries, TM has been an integral resource for health in households and communities. The WHO recognizes the value and diversity of the cultures of Indigenous Peoples and local communities, along with their traditional knowledge. To attain the health-related Sustainable Development Goals (SDGs) and the WHO's Triple Billion Targets, which aim to ensure universal health coverage, protection from health emergencies, and improved well- being for over one billion people, concerted efforts are being made. The WHO is committed to exploring ways to integrate evidence-based traditional and complementary medicine services, particularly for primary health care, to achieve UHC and ensure healthy lives and well-being for all. The WHO global report on Traditional and Complementary Medicine 2019 and the WHO Traditional Medicine Strategy: 2014–2023 remain valuable resources for governments, system planners, and health practitioners[2]. In this editorial, we use the broader concept involving IK and Traditional, Complementary, and Integrative Medicine (hereinafter referred to as TCIM) as a holistic system for planetary health and well-being.

Numerous health-seeking behavior studies indicate that over 80% of people from both low and high-income countries want to use TCIM for their health-related problems. The WHO has received requests from member states to integrate TCIM with conventional healthcare and provide evidence and data to inform policies, standards, and regulations for its safe, equitable, and cost-effective use.

Consequently, the 76th World Health Assembly in May 2023 resolved to develop a new WHO Global Strategy for Traditional Medicine 2025–2034, recognizing the benefits of TCIM demonstrated in managing various health conditions including the COVID-19 pandemic.

TCIM for global health

In the current healthcare scenario, the experiential wisdom of IK/TCIM systems can be a valuable ally in providing a holistic and culturally sensitive approach to healthcare. Integrating TCIM into conventional healthcare systems has the potential to promote universal well-being, affordability, access, and equity in line with the principles of planetary health. TCIM systems encompass a diverse range of traditional healing practices, including: Indian Ayurveda, Yoga, Unani, Siddha, Sowa Rigpa, Naturopathy, Homeopathy, and herbal medicine; and various other indigenous healing systems worldwide such as African, European, American aboriginal, Australian Bush, Indonesian Jamu, Malay, Māori, Persian, Tibetan, Thai, and Traditional Medicine of East Asia (Japanese Kampo, Korean and Traditional Chinese Medicine) just to mention a few. By embracing the diversity of traditional healing practices, we can create a more inclusive and equitable healthcare system that values the experiential wisdom and knowledge of different cultures and societies

TCIM focuses on prevention and lifestyle interventions aligning with the SDGs' targets on health promotion and disease prevention. Herbal medicine, fasting, forest and nature-based therapies, and practices like Yoga, Tai Chi, Qigong, and mindfulness meditation have been used for centuries to strengthen the body's resistance and enhance resilience. Prioritizing prevention empowers healthy living, good nutrition, coping with stress reducing chronic diseases, and enhancing well-being. In contrast to the typically reductionist approach of conventional medicine, TM emphasizes the interconnectedness of mind, body, and spirit, taking into consideration the relationship between the planetary environment and the social and political systems in which we live.

Mainstreaming TCIM into primary healthcare systems can bridge access gaps, making healthcare more inclusive, culturally sensitive, and equitable. This, in turn, can foster sustainable development by empowering local communities, supporting traditional healers, and promoting eco-friendly healing practices. TCIM also supports local herbal medicine industries, traditional healing centers, and wellness tourism, thus stimulating economic growth in rural and marginalized areas, and contributing to sustainable development and poverty reduction.

Evidence-based integration

Evidence-based integration is vital in bridging the gaps in conventional healthcare which is more about symptomatic care based on the diagnose-dispense-refer model. More attention should be given to patient education and addressing the root causes of illness which might have historical, social, nutritional, and environmental origins. TCIM approaches can facilitate the shift from a reactive sick-care approach to proactive preventive measures, leading to better health outcomes in the long run. Conventional medicine demonstrates proficiency in areas such as: in diagnostics, emergency care, surgeries, infection management, and symptomatic relief through powerful pharmaceutical drugs. On the other hand, TCIM distinguishes itself with its person-centered approach, emphasizing a holistic view that considers the interplay of mind, body, and environment. It focuses on building natural resilience, adaptability, and managing non- communicable and psychosomatic chronic conditions. Combining the strengths of both systems enables a more comprehensive and personalized approach to healthcare.

TCIM integration supports cultural preservation, acknowledging the importance of traditional knowledge and wisdom. TCIM systems also offer additional tools to address emerging health challenges, including antimicrobial resistance and

emerging infectious diseases. Building global partnerships and sharing best practices in TCIM can foster cross-cultural understanding and collaboration, leading to a more inclusive and culturally sensitive approach to healthcare. It is possible that by embracing the principles of TCIM and integrating them into mainstream healthcare, we can promote affordability, accessibility, and better health outcomes for all. It is time for a paradigm shift from a disease-oriented approach to a holistic healthcare model that respects the diversity of healing traditions and empowers individuals and communities to take charge of their health and well-being. With evidence-based integration and collaborative efforts, TCIM can lead to a healthy and sustainable future for the next generations.

Undoubtedly, the TCIM systems play a crucial role in global public health, planetary health, holistic well-being, and equitable healthcare. To ensure successful integration, research using appropriate methods is imperative. Equally important are fair mechanisms for protecting intellectual property rights, benefit sharing, and promoting equitable access to TCIM. Initiatives such as Traditional Knowledge Digital Library (TKDL) from the Government of India remain useful in this direction.

Collaborations between TCIM practitioners, conventional medical professionals, and researchers can pave the way for evidence-based integration. While the integration of TCIM holds immense promise, it also presents challenges that need to be addressed. Along with evidence of efficacy, assurance of safety, quality, standardization of practices, products, training of practitioners, pharmacovigilance, regulatory controls, and ethical considerations are key areas requiring attention among others. Collaboration and dialogue between governments, healthcare professionals, and local communities on policy frameworks for integration of TCIM is necessary.

WHO Global Center for Traditional Medicine

In this context, we commend the WHO leadership, particularly Director-General Dr. Tedros Adhanom Ghebreyesus, for his unwavering commitment to engaging all stakeholders, including governments, experts, and civil society organizations (CSOs) in meaningful consultations. Dr. Tedros' acknowledgment of Traditional Medicine's deep-rooted knowledge and resources in communities reinforces TCIM's integral role within health systems worldwide, providing vital support to millions of people. Recognizing the growing global interest in TCIM, the WHO, with the generous support of the Government of India, has established the Global Centre for Traditional Medicine (GCTM) in Jamnagar, Gujarat, India. Aligned with the Indian philosophy of Vasudhaiva Kutumbakam (One World-One Family-One Future), the GCTM aims to be a beacon of knowledge, combining ancient wisdom with modern science to catalyze transformative progress for the health and prosperity of humanity and the planet[3]. Its multidimensional approach focuses on evidence and learning, data and analytics, sustainability and equity, and innovation and technology to underpin global health and sustainable development.

As a noteworthy stride towards achieving health and well-being for all, the GCTM is organizing the first WHO Traditional Medicine Global Summit (TMGS) on August 17–18, 2023, in Gandhinagar, Gujarat, India. Co-hosted by the WHO and the Government of India, and co-branded with the G20 Ministers meeting, this landmark event will serve as a platform for policymakers and stakeholders to share best practices, innovations, data, and scientific evidence on the invaluable contributions of TCIM to global health, well-being, and sustainable development. The Summit will set a precedent for international health discourse, fostering cooperation among nations and transcending geographical boundaries. With a focus on advancing traditional medicine to the forefront of healthcare strategies, the Summit will forge new partnerships, ignite innovative ideas, and cultivate a shared commitment to achieving health and well-being for all.

As the GCTM embarks on a mission and strategic plan towards a healthier and more sustainable future, we express gratitude to the WHO and the

Government of India, Ministry of Ayush for their leadership and generous support in establishing this transformative force for global good. Together, as a global family, we honor the legacy of ancient wisdom and acknowledge its impact on our present and a pivotal role in shaping our future. The establishment of the GCTM and the Summit is a milestone exhibiting reinforcement of our commitment to scientific development in TCIM systems for global health and well-being.

Pivotal recommendations

At this historical juncture, the WHO Expert Advisory Group proposes five pivotal recommendations: First, harness the contribution of TCIM to advancing planetary health and well-being at all ages by ensuring regional and culturally appropriate nutrition and lifestyle for a sustainable environment and promoting a well-being economy framework recognizing the value of indigenous knowledge that stood the test of time. Second, encourage more transdisciplinary research, develop appropriate methods, and enhance research funding to TCIM for innovation, technology, and translation commensurate with public demand and use. Third, strengthen strategies for information, education, and communication to create more awareness about the importance of scientific evidence, and promote understanding and trust among all stakeholders in multi-sectoral partnerships to co-develop international standards and accreditation systems for practice, regulation, and to advance knowledge from TCIM. Fourth, redefine laws, policies, and health services to enable holistic, informed, seamless choices with a transformative focus on prevention, primary care, planetary health, and well-being rather than simply distributing integrated cure services and interventions. Fifth, shift political and economic models beyond mere profits to promote equity of access, rights, shared benefits, financial protection, and incentives to make TCIM a driving force in achieving health-related SDGs, planetary health, and well- being for all.

Towards planetary health and well-being

The evolution in medical systems over the past few decades has led from eminence-based medicine to evidence-based medicine, and transmuting to increasingly patient-centered medicine. With more focus on preventive and resource- oriented medicine considering the entirety of humans and the ecosystem in the sense of One Health, planetary health, and well-being, TM offers approaches for the promotion of health and well-being. In several regions, experiences of evidence-based integration of TCIM with conventional healthcare during the COVID- 19 pandemic have been very encouraging [4]. This is the time to break ‘pathy’-based silos to transgress from the EGO system to the ECO system prioritizing mutual respect, public needs, and global good [5]. We may envisage the integration of TCIM and conventional medicine as a modern healthcare system in the future.

We are confident that the WHO GCTM and the first TMGS will stimulate cross-cultural, transdisciplinary, intellectual dialogue converging into a pathbreaking declaration. The Summit can also enable the global community to a resilient health system integrating evidence-based, patient-centered healthcare systems for more inclusive, culturally sensitive, and equitable healthcare. With concerted efforts and a shared vision, we can harness the immense potential of time-honored healing traditions to transform lives and our planet.

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Author contributions

Bhushan Patwardhan conceptualized and created the first draft. All the Members of the WHO External Advisory Group for TMGS endorsed the idea and the final manuscript. L Susan Wieland, Anchalee Chuthaputti, Roshanak Ghods, Goh Cheng Soon, Georg Seifert, Sione Tu’itahi, Ricardo Ghelman, Shyama Kuruvilla, Kim Sung Chol, and Kathi Kemper critically reviewed the draft, added content,

and offered valuable suggestions for improvement. Participating Editors reviewed, endorsed and agreed to publish it online/in print.

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Text footnote

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Clinical evaluation of *Dashangalepa* formula for pain and swelling of joint diseases with *in-vitro* anti-inflammatory activity

Jayasiri A.P.A.[1]*, Amarasinghe A.P.G.[2], Paranagama P.[3] and Senanayake S.P.[4]

Abstract

Joint diseases are categorized into different types based on their symptoms in Ayurveda, predominant symptoms with pain and swelling, loss of activity, present deformities. Among them *Sandigatha vata* (osteoarthritis) and *Amavata* (Rheumatoid arthritis) are very common conditions. *Dashangalepa* is used as topical application to manage acute and chronic painful inflammatory musculoskeletal conditions. Aim of this study to evaluate the efficacy of reducing pain and swelling with anti- inflammatory activity. Volunteer Sixty (60) patients with *Sandigatha vata* or *Amavata* were selected and divided into two groups (n=30). Group I and II apply the test drug *Dashangalepa* and reference drug Rumalaya gel respectively. Pain was evaluated using universal pain assessment tool and swellings of the joints were taken by using tape measurements, in every 14 days for 8 weeks. Biochemical investigations were estimated for ESR, RH-factor, C-reactive proteins, SGOT, SGPT. Statistically significant reduction ($P< 0.05$) was observed in symptoms treated with test drug. Biochemical parameters not showed any alterations in pre and post treatment stages, which confirmed its safer efficacy. In vitro anti- inflammatory activity of *Dashangalepa* aqueous acidic preparation and Rumalaya gel were assessed separately using Human Red Blood Cell membrane stabilizing activity method, results revealed activity of both test and reference drug are similar and activity is increased by increasing the concentrations. Results can be concluded that test drug is similar herbal formula to previously proven reference drug, with safer effective anti-inflammatory pain and swelling reducing herbal formula for treat joint diseases.

Keywords: Joint diseases, pain and swelling, anti-inflammatory, topical application

Introduction

Joint diseases are described in different aspects in Ayurveda medicine, as per the concepts vitiated doshas comes to the joint and caused pain and swelling of that joint, that causes displace the joint and disturbed the activity of that particular joint. In Ayurveda system of medicine joint diseases are categorized into different types according to their symptoms[1]. They are pain predominant diseases (*Shoola pradhana*), swelling predominant diseases (*Shotha pradhana*), pain and swelling predominant diseases (*Shoola, Shotha pradhana*), diseases predominant loss of activity (*Kriya hani pradhana*) and deformities predominant diseases (*Vikurthi pradhana*)[2]. Among the several joint diseases *Sandigatha vata* (osteoarthritis) and *Amavata* (Rheumatoid arthritis) are very common conditions symptomatically present pain and swelling in patients. Causative factors for these diseases are age factors, structural changes due to the inflammation, free radical activity, and behavioral changes as well as climatic conditions.

About 1% of the world population is afflicted by rheumatoid arthritis, women are affected 3 times than man, rheumatoid arthritis occurred throughout

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the world and all the ethnic groups, climates, altitude and geography[3]. Osteoarthritis (OA) is more generalized and more serve in older women. It is estimated that approximately 50% of the world population over 65 years of age is affected by osteoarthritis[4]. Osteoarthritis is characterized by joint pain and tenderness, limitation of movements, crepitus, occasional effusion and variable degree of inflammation without systemic effects[5].

Inflammation is considered as a primary physiological defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli[6]. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases[7]. The drugs used in the treatment of rheumatoid arthritis act rapidly, relieve pain and control inflammation, and help to improve and maintain joint function and to prevent deformities.

Dashangalepa (DL) is a polyherbal drug preparation which is used for edematous conditions as topical application to manage acute and chronic painful inflammatory musculoskeletal conditions in Ayurveda system of medicine[8,9]. Furthermore, topical applications offer short and long term safety from adverse events such as burning, stinging and erythema because topical applications are mainly limited to the site of application[10].Therefore it is important to develop and evaluate the efficacy of topical applications practice in Ayurveda clinical practices. Clinical studies have proven the benefits of topical analgesics, in the management of certain acute and chronic painful inflammatory musculoskeletal conditions[11]. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti- inflammatory drugs with fewer side effects is necessary. Therefore, this attempt has been taken to evaluate the efficacy of *Dashngalepa* formula with its anti-inflammatory effect.

Materials and methods

Selection of patients

Volunteer Sixty (60) patients with clinical symptoms of pain and swelling in *Sandigatha vata* (osteoarthritis) or *Amavata* (rheumatoid arthritis) were included from selected Ayurveda hospitals of Sri Lanka. Ayurveda Teaching Hospital Borella, Bandaranayake Memorial Ayurveda Research Institute, Navinna, in western province and Ayurveda base Hospitals of Ratnapura, Kegalla and Embilipitiya in Sabaragamuwa province of Sri Lanka after the approval of the Ethical Review Committee of Institute of Indigenous Medicine, University of Colombo, (ERC 12/01) 2012. The patient whose age was between 20-80 years of both sexes and the patients who were having signs and symptoms of pain and swelling were included to the study and patients with renal, hepatic or cardiac problems, bone fractures, pregnant and nursing mothers were excluded from the study. A detailed clinical examination and relevant laboratory investigations were conducted and all required information were documented in a specially prepared performa with written consent of the patients.

Preparation of drug

Dashangalepa herbal formula was prepared according to the *Lepa* (external applications) preparation method of Ayurveda Pharmacopeia. Shade dried ten ingredients of *Dashangalepa Albizzia lebbeck* (stem bark), *Glycerhizzia glabra* (stem parts), *Valeriana wallchi* (whole plant), *Pterocarpus santalinus* (heart wood), *Elataria cardamomum* (seeds), *Nadostachus jatamansi* (root), *Curcuma longa* (rhizome), *Cosinium fenestratum* (stem parts), *Saussurea lappa* (roots) and *Plectrathus zeylanicus* (whole plant) were finely powdered separately, using a mechanical grinder (Disk Mill, SHC 23, China). Equal amounts (10g) of powder of ten ingredients of the *Dashangalepa* topical application were mixed together and prepared the powder form of *Lepa* (paste).

Selection of reference drug

Rumalaya gel (R) polyherbal formula was taken as the standard reference drug for the clinical study. Rumalaya gel has analgesic, anti-inflammatory, antioxidant, counterirritant, glycosaminoglycan building and cartilage healing properties. It also has vasodilatation of cutaneous vasculature, which increases blood circulation and produces a feeling of warmth. Consequently, cutaneous receptors are stimulated for thermal sensations, which serve to distract deep seated pain sensations[12].

Ingredients of Rumalaya gel are *Menthaavensis (Pudina*), *Gaulthera fragrantissima (Gandhapura taila), Pinusroxburghii (Sarala), Cinnamomum zeylanicum (Thvak), Cedrus deodara (Badradaru), Vitex negundo (Nirgundi), Boswella serrata (Shallaki)* and *Zingiber oficinale (Shunti).*

Administration of drug

The patients were divided into two groups (n=30), test drug DL (group I) and R (group II) were distributed among them. Test drug of 100g packets were given and advised them to convert the powder into a paste according to the following instructions. Put the sufficient amount of powder into a clay pot and mixed with fresh juice of leaves of *Tamarindus indicus* until cover the amount of powder. Then convert it into a semi solid paste by application of mild heat. Apply the paste over the swollen joints with pain and swelling once day, and kept it for eight hours, for 14 days. Patients were instructed to apply Rumalaya gel amount need to cover the area of swelling from the 30gram of tube as a topical application once a day and kept it until dries up, for 14 days. Symptomatically improvement of pain and swelling of each group was recorded.

Assessment criteria

Symptomatically improvement of pain and swelling of each group was recorded using clinical performa once in every 14 days for 8 weeks. Biochemical investigations included ESR, RH-factor, C-reactive proteins, SGOT, SGPT were performed before and after the treatment. Joint pain was evaluated according to visual analogue scale using universal pain assessment tool13,14,15 as described here [(0-no pain), (1-mild pain= can be ignored), (2-moderate pain=interferes with tasks/sleep),(3-severe pain= interferes with basic needs)]. Evaluation of swelling of the joints were done by using tape measurements16. Follow up was done after 8 weeks of treatment period for two months of duration in every 14 days.

Statistical analysis

The reduction in pain and swelling score were evaluated comparing to standard reference drug. Efficacy of DL preparation comparatively with the reference drug was evaluated by using swelling measurements and pain reduction scale. Paired sample t- test was used with 95% confidence interval to analyze the efficacy of this preparation.

Experimental evaluation of *Dashangalepa* formula

Preparation of acidic aqueous extract of *Dashangalepa* for anti-inflammatory activity

The powdered preparation of *Dashangalepa* (100 g) mixture was added to 400 mL of fresh juice of Tamarindus indicus (*Siyabala)* leaves and kept on the shaker for six hours. The mixture was filtered and the filtrate was freeze dried using a freeze dryer (Labconco, cat. 01, Missouri). The weight of the extract was recorded.

Investigation of in vitro anti-inflammatory activity of aqueous extract of Dashangalepa and Rumalaya gel

In vitro anti- inflammatory activity of DL aqueous acidic preparation and Rumalaya gel were assessed separately using HRBCMS (Human Red Blood Cell membrane stabilizing activity) method as described previous research17. From the test samples of the drug preparation (DL) and (R) (1 mg / mL) were dissolved in 0.2 mL of DMSO and diluted to 5 mL by adding appropriate amount of normal saline. The reaction mixture was prepared with 5 mL of test solution and 0.5 mL of 10 % RBC suspension. The negative control was prepared using equal amount of DMSO and normal saline (Figure 1). All the centrifuge tubes containing reaction mixtures were incubated in a water bath at 56^{o}C for 30 min and the

tubes were cooled under running tap water (Figure 2). The reaction mixtures were centrifuged at 3000 rpm for 10 min and the absorbance of the supernatants were taken at 560 nm using UV-Visible spectrophotometer (Aqua Mate 8000, Singapore). The test sample was performed in triplicates and the percentage stability was calculated using the equation (1) mentioned below.

$$***\%\ Stability\ = \frac{A_{control} - A_{sample}}{A_{control}} \text{--------} (1)$$

Figure 1 and 2 shows the HRBCM stability assay conducted for anti-inflammatory activity

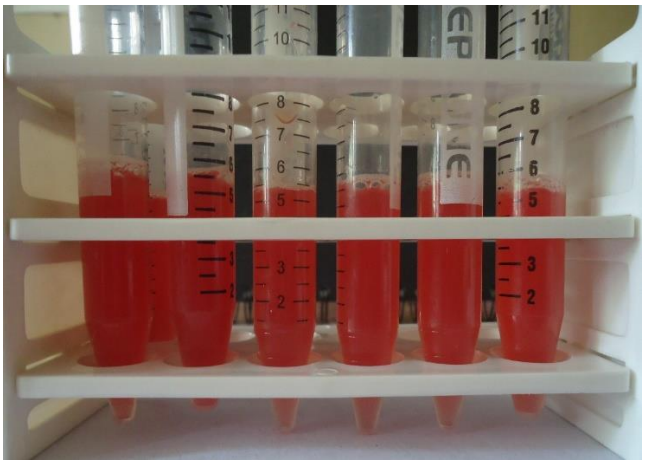


Fig. 1: With different concentrations of DL extract before incubation

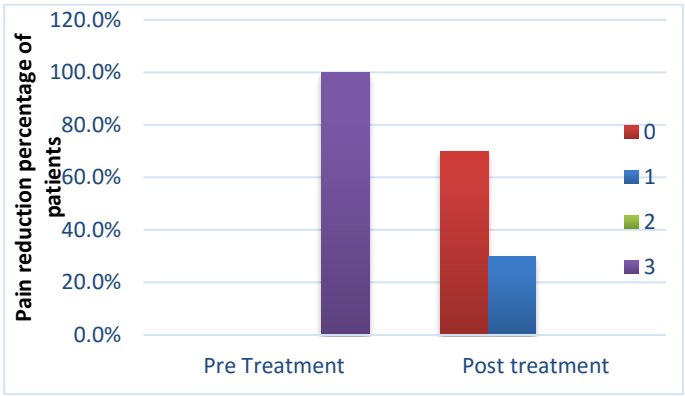
Fig. 2: With different concentrations of DL extract after incubation

The percentage stability versus test concentrations were plotted in order to compare the anti-inflammatory activity with the standard drug separately. The IC50 of the samples were calculated using Probit analysis (MINITAB® Release 14.2 Minitab Inc. 2003 statistical software).

Results

Observations of the clinical study

Total sixty patients were completed this trial, among them age incidence of the cases registered for this study belongs to the age group between twenty to eighty years. Hundred percent (100%) of patients in pretreated stage included to severe pain category of the group treated with the DL preparation. The observations revealed pain reduction as a percentage was reduced up to 65% (no pain), 35% (mild pain) are shown in Figure 3 at the stage of post treatment. There was no any patient remain in severe or moderate pain categories. These results revealed DL is an effective preparation. Pain reduction of post treated stage in group of DL preparation demonstrated significant change at the level of 95% confidence interval of the difference (P< 0.05).

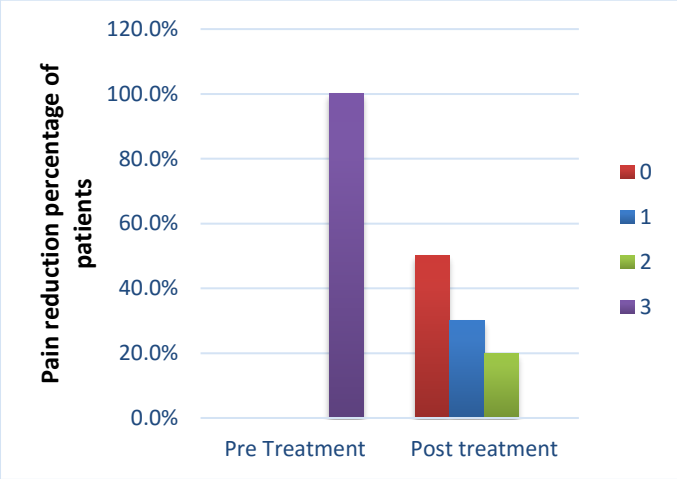


0 - no pain 1 – mild pain 2 – moderate pain 3 – severe pain

Fig. 3: Effectiveness of *Dashangalepa* on pain of the arthritis patients (n = 30)

Hundred percent (100%) of patients in pretreated group of Rumalaya Gel included severe pain category, shown at post treatment stage shown the percentage number of patients in that group reduced to 50%, 30%, and 20% no pain, mild pain, moderate pain respectively are demonstrated in Figure 4.

There were no any patient remain in severe pain category. Pain reduction of post treated group of (R) observed significant change at the level of 95% confidence interval of the difference (P< 0.05).



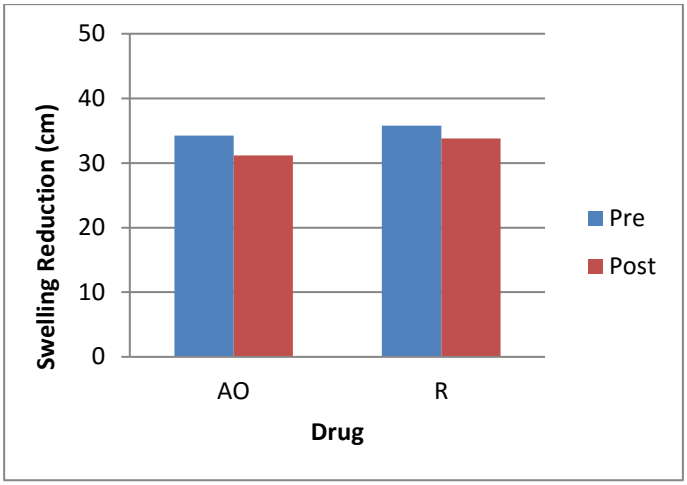
0 - no pain 1 – mild pain 2 – moderate pain 3 – severe pain

Fig. 4: Effectiveness of Rumalaya gel on pain of the arthritis patients (n = 30)

Reduction of swelling

Swelling reduction among DL compares to the standard drug Rumalaya gel in pre-treatment and post treatment stages were recorded considering the tape measurements. Pre-treatment and post treatment mean values of swelling measurements of standard drug Rumalaya gel are 36cm and 34cm respectively. The swelling reduction between pre and post treatment 2cm is shown in Figure 5.

Test drug DL mean value of swelling reduction of pre and post treatment was 34 cm and 31 cm respectively and the difference of swelling reduction between pre and post treatment is 3 cm demonstrated in Figure 5.



DL – Dashangalepa R- Rumalayagel Pre – Pre-treatment Post – Post treatment

Fig. 5: Effectiveness of DL and Rumalaya gel on swelling of the arthritis patients (n = 60)

Evaluation of biochemical parameters

Evaluation of biochemical parameters ESR, Rheumatoid Factor, C– reactive Protein, SGOT and SGPT no significant alterations were seen and they were demonstrated in Tables 1 and 2.

Results of bio chemical parameters; ESR, Rh factor, CRP, SGOT and SGPT not shown any alterations in pre and post treatment, it was not significant change in biochemical parameters.

Evaluation of Anti- inflammatory activity of Dashangalepa preparation

Results of Red Blood Cell Membrane stabilizing activity of *Dashangalepa* formula and standard drug R are presented in Figure 5. The results indicate that anti-inflammatory effect of DL preparation was similar to that of Rumalaya gel.

According to the results of membrane stabilizing activities of RBCM, it was shown that the activity is increased by increasing the concentration of *Dashangalepa* preparation and Rumalaya gel.

Results of bio chemical parameters

ESR, Rh factor, CRP, SGOT and SGPT not shown any alterations in pre and post treatment, it was not significant change in biochemical parameters.

Table 1: Biochemical Investigations of DL_4 treated group (n = 30)

Parameter	Pre-Treatment	Post Treatment	P value	Significance
ESR	49.06 ± 28.6	40.46 ± 29.5	0.000	NS
Rh Factor	1.37 ± 0.5	1.27 ± 0.5	0.184	NS
CRP	7.38 ± 12.8	7.79 ± 13.8	0.545	NS
SGOT (IU/L)	15.18 ± 6.4	15.57 ± 7.2	0.368	NS
SGPT (IU/L)	15.07 ± 7.6	14.74 ± 6.5	0.469	NS

Table 2: Biochemical Investigations of R treated group (n = 30)

Parameters	Pre-Treatment	Post Treatment	P value	Significance
ESR	37.7 ± 18.4	31.13 ± 17.41	0.001	NS
Rh Factor	1.17 ± 0.3	1.13 ± 0.3	0.326	NS
CRP	3.6 ± 4.6	2.8 ± 1.9	0.272	NS
SGOT (IU/L)	14.75 ± 6.1	14.94 ± 6.2	0.469	NS
SGPT (IU/L)	14.16 ± 5.1	14.96 ± 5.3	0.024	NS

Evaluation of Anti- inflammatory activity of Dashangalepa preparation

Results of Red Blood Cell Membrane stabilizing activity of *Dashangalepa* formula and standard drug R are presented in Figure 6. The results indicate that anti-inflammatory effect of DL preparation was similar to that of Rumalaya gel.

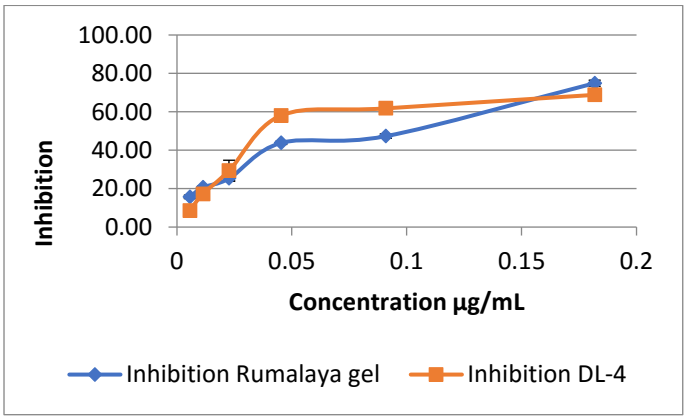


Fig. 6: Red Blood Cell membrane stabilizing activity of Dashangalepa preparation and Rumalaya gel. Each value is expressed as mean ± SD (n=3).

According to the results of membrane stabilizing activities of RBCM, it was shown that the activity is increased by increasing the concentration of *Dashangalepa* preparation and Rumalaya gel.

Erythrocyte membrane is structurally analogous to the lysosomal membrane. The IC_{50} (50 % Inhibition concentration) values obtained for anti-inflammatory assays are used for the interpretation of the results that is defined as the concentration of the test sample required to stabilize 50 % the red blood cell membrane where the response is reduced by half. Stabilization of lysosomal membrane is important in limiting the inflammatory response of reactive species[18]. Therefore, the use of erythrocyte membrane is a good model to study the protective effect of herbal products for evaluate Anti-inflammatory activity as an in-vitro evaluation method[19].

EC_{50} values of the tested preparations DL and R were estimated using Probit analysis (MINITAB® Release 14.2 Minitab Inc. 2003 statistical software) the results were given evidence of that similar EC_{50} values of and DL and Rumalaya gel 0.13 ± 0.21, 0.11 ± 0.02 (µg /mL) respectively indicate the effects of these preparations were similar to each other. The lower EC_{50} values demonstrated the higher anti– inflammatory activities that give beneficial for this product.

Discussion

According to the pharmacological properties describe in Ayurveda *Dravyaguna Vignana* this formula contained best *Vedana sthapana* (pain reliever) and *Shotha hara* (eradicated edema) drugs *Albizzia lebbeck, Glycerhizzia glabra, Valeriana wallchi, Pterocarpus santalinus, Nadostachus jatamansi, Curcuma longa, Cosinium fenestratum, Saussurea lappa* as ingredients[20].

Both diseases investigated in this study are pain (*Vedana /Shoola*) and swelling (*Shotha*) predominant diseases. In Ayurveda texts they have stated *A. lebbeck* (AL) is *Vedana sthapana* (pain reducing) and *Shotha hara* (swelling reducing) drug material[21]. Rareness of AL many other substitutes were used for this DL formula instead of (AL), therefore This clinical study with experimental part designed to evaluate the efficacy of DL prepared with *Albizzia lebbeck* original plant in Sri Lanka to reduce the pain and swelling of joint diseases that main symptoms complain by the patients.

Erythrocyte membrane is structurally analogous to the lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response of reactive species. Therefore, the use of erythrocyte membrane is a good model to study the protective effect of medicinal plant extracts and the herbal products. The ingredient of this formula *Albizzia lebbeck* shown its solvent and aqueous extracts already proven for Anti - inflammatory activity[22].

When considering the causative factors for this type of degenerative changes of the bony structures defense mechanism such as anti –oxidants are very important for drug evaluation with their discoveries. Many research papers have been published on the antioxidant properties of *Albizia* species[23]. reported the antioxidant activity of *A. julibrissin* and in their study it was reported that the bark extract contained quercetin derivative, hyperoside and quarcitrin which contributes to the higher antioxidant activity. As AL is included to the same genus it also has the trend to constitute with similar chemical compounds that may help to show the certain efficacious results. Antioxidants are the health protective compounds that responsible to neutralize the free radical formation in the body which destruct the body tissues same way effect on the bony structures regarding to the bone and joint diseases. Antioxidant activity of the bark extract of *A. lebbeck* has been studied by using DPPH and reducing power already proven its anti-oxidant power[24].

This study confirmed swelling reduction efficacy of drug preparation effective than the standard reference drug Rumalaya gel for reducing swelling in arthritis patients. Certain other ingredients of the preparation such as *Curcuma longa* and *Glycyrrhizaglabra* which were known as anti-inflammatory herbs[25,26].

Earlier research proved plant extracts exerted maximum membrane stabilizing activity to standard anti- inflammatory drugs. The mode of action of the extract of the formula and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual like repulsion charges which are involved in the haemolysis of red blood cells. Research reports were proven that methanol and aqueous extracts of plants presence of saponins and tannins aided to exhibit the anti-inflammatory activity [27]. Earlier research reported ingredient AL was positive for saponin[28] and also reported significant anti-inflammatory effect can be induced due to the presence of glycosides or steroids[29]. Present study also exhibited freeze dried aqueous extract of DL have red blood cell membrane stabilizing activity was demonstrated well. These findings confirmed that this preparation can be developed as anti-inflammatory value-added product to treat arthritis patients in Ayurveda system of medicine.

Earlier research reported Rumalaya gel has the beneficial effect for heal arthritis patients due to the synergistic actions of its ingredients. Rumalya gel has analgesic, anti- inflammatory, antioxidant, counterirritant, glycoseaminoglycan- building and cartilage healing properties. Rumalaya gel induces vasodilation of cutaneous vasculature, which

increases blood circulation and produces a feeling of warmth. Consequently, cutaneous receptors are stimulated for thermal sensations, which serve to distract deep-seated pain sensations. Present study shown that comparable efficacy of DL to the standard drug Rumalaya gel due to the presence of chemical constituents which are responsible for defend inflammatory process, same manner DL also act as the reference drug R gel.

Conclusion

In this study there was an excellent relief of pain and reduction of swelling at the end of the therapy. Also it is comparable in activity to the already proven reference drug Rumalaya gel. Biochemical analysis gives evidence in safety of this formula that not shown any significant increase of bio chemicals. Results of this study provide a good working base for future workers, especially for those who work along the lines of drug actions and their active chemical constituents. Anti-inflammatory effect of this formula is good outcome of this study to develop novel herbal topical applications for further research with value addition to the conventional *Lepa* formulations.

Acknowledgement

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Efficacy and safety of virgin coconut oil and king coconut oil compared to liquid paraffin as a moisturizer for mild atopic dermatitis: A pilot study

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Abstract

Atopic dermatitis is a chronic skin condition due to altered skin barrier. There is no cure for the condition, but it can often be managed with proper care and treatment. Application of a moisturizer is a mainstay of treatment to manage the condition. Therefore, this study assessed the effectiveness and safety of virgin coconut oil (VCO) and king coconut oil (KCO) compared to liquid paraffin as a moisturizer for mild atopic dermatitis. The study was conducted as a pilot study of a randomized, double-blind, parallel group comparison trial on patients with mild atopic dermatitis. Patients were randomized to receive VCO, KCO or liquid paraffin. The outcome measures were SCORing atopic dermatitis (SCORAD) index and Patient Oriented Eczema Measure (POEM) score and instrumental measurements of skin moisture and skin lipid levels at two weeks intervals for 3 times. The results showed a significant improvement of eczema was seen in relation to SCORAD index and POEM scores in all three arms. Significant improvement of moisture levels was seen in KCO and liquid paraffin arms. However, no improvement of lipid levels was seen in all three arms. It was concluded that, this pilot study shows that VCO, KCO and liquid paraffin are equally effective moisturizers for the treatment of mild atopic dermatitis. VCO and KCO can be used instead of hydrocarbon-based liquid paraffin in the treatment of mild atopic dermatitis as those vegetable oils are relatively inexpensive and widely available in Sri Lanka.

Keywords: atopic dermatitis, virgin coconut oil, king coconut oil, liquid paraffin

Introduction

Atopic dermatitis (AD) is a chronic pruritic skin disease that occurs due to defects in the epidermal barrier function and cutaneous inflammation[1]. The lesions have ill-defined erythema, with oedema, and vesicles which are predominantly seen in skin flexures. In the chronic stage of AD, the skin becomes lichenified[2]. AD can occur at any age, but the prevalence is high in children[3].

There are several aetiological factors in AD. They include genetic factors, allergens like house dust mites, foods, *Staphylococcus aureus* infections and exposure to excessive heat and irritants[3]. Due to the defects in epidermal barrier function, AD patients are more prone to allergic sensitization, microbial colonization and infections[4.5]. When the epidermal barrier function is defective trans-epidermal water loss (TEWL) will be increased and water retention capacity will be reduced leading to dry skin. There are low levels of skin lipids and ceramides in patients with AD[6].

The diagnosis of AD is essentially clinical. The Hanifin and Rajika diagnostic criteria for AD are widely used to confirm the diagnosis of AD for research purposes[2].

Moisturizers should be considered as the mainstay of AD management[7].

With the use of moisturizers, dry skin, itching and the penetration of skin by irritants and allergens are reduced leading to a significant improvement in appearance and the symptoms of dry skin[2.8]. Moisturizers have ingredients to achieve hydration and improve skin barrier properties such as occlusives, humectants and emollients[9]. Humectants

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are water-attracting substances with a low molecular weight[10]. Occlusive prevent trans-epidermal water loss (TEWL) by forming a hydrophobic barrier over the skin. Emollients fill the gaps and fissures and help to improve the skin texture[11].

The inflammatory component of AD is usually managed with the use of corticosteroids[8] Antihistamines are often used for symptomatic relief.

The use of moisturizers has been shown to enhance the response to treatment with topical corticosteroids in AD[12].

Topical calcineurin inhibitors such as tacrolimus and pimecrolimus also can be used as other treatment options.

In selected patients, antibiotics, systemic immuno-modulators and ultra-violet light are used[2].

Skin is composed of two main layers known as epidermis and dermis. The epidermis prevents water loss from the skin and acts as a protective barrier for various allergens. The stratum corneum is the protective outer layer that is responsible for preventing TEWL.

The most significant risk factor for AD is mutation in the human filaggrin gene (FLG) which codes for skin barrier protein filaggrin[13]. Filaggrin is a major structural histidine-rich protein localized in the stratum corneum of the skin. Filaggrin aggregates filaments catalyzing the formation of disulfide bonds between keratin fibers. These aggregated fibers maintain the flattened shape characteristic of corneocytes. Once the keratin fibers are formed, filaggrin is degraded.

The natural moisturizing factor (NMF) components in the corneocytes are the breakdown products from the proteolysis of filaggrin. NMF components act as humectants and keep the corneocytes hydrated by attracting and binding to atmospheric water[14]. The degradation process of filaggrin depends on the water content within the corneocytes. If the water content is low the hydrolytic enzymes will not be able to degrade filaggrin and therefore the levels of NMF are reduced[15].

Decreased levels of filaggrin lead to increased TEWL and the reduction in stratum corneum hydration[16]. The softness and flexibility of the stratum corneum are maintained by water and when there is reduced water, the stratum corneum becomes hard and brittle[17].

Lipids that are found in the stratum corneum help to maintain the water barrier of the skin as well as help to retain NMF within the corneocytes. Lipid levels in the stratum corneum vary with factors such as genetic variation, age, diet and environmental factors. Therefore, reduced lipid levels in the stratum corneum may predispose to dry skin[14].

Various natural and synthetic agents are used as moisturizers to improve the skin barrier function.

Virgin coconut oil (VCO) is a safe, non-toxic, natural moisturizing agent used in the traditional systems of medicine. VCO is extracted from the fresh and mature kernel of the coconut[18]. VCO is colourless and has the aroma of fresh coconut. It can be used for cooking and cosmetic purposes.

VCO has many clinically useful phytochemicals and physicochemical properties[19].

VCO consists of triglycerides >99% and it is rich in medium-chain fatty acids. The chemical composition of VCO is shown in Table 1.

Table 1: The chemical composition of VCO

Fatty acid	Lipid number	Composition %
Caproic acid	C6:0	0.2
Caprylic acid	C8:0	7.8
Capric acid	C10:0	6.5
Lauric acid	C12:0	50.6
Myristic acid	C14:0	19.5
Palmitic acid	C16:0	6.5
Stearic acid	C18:0	1.9
Oleic acid	C18:1	4.5
Linoleic acid	C18:2	2.5

In addition to triglycerides, VCO contains phenolic acids and polyphenols that have antioxidative, anti-inflammatory, antibacterial, and wound-healing properties[20].

VCO, by forming an occlusive film on the skin, significantly reduces TEWL and improves skin hydration[1]. The anti-inflammatory effect is provided by the medium-chain fatty acids (produced as a

result of the degradation of triglycerides by the lipases of skin flora), phenolic acids and polyphenols of VCO[1].

The antibacterial effects of VCO are mainly due to monoglycerides. *Staphylococcus aureus* in infected lesions of AD produces lipases that hydrolyse triglycerides in VCO to monoglycerides. Monoglycerides and medium-chain fatty acids in VCO prevent bacterial growth[20].

A study on the in-vitro anti-inflammatory and skin protective properties of VCO has found that topical application of VCO increases the filaggrin level in corneocytes and improves the barrier function (21). This finding supports the use of VCO to treat AD, in which defective barrier function is a major contributory factor[21].

Another study has shown a 68.23% reduction in the SCORAD index and an improvement of post-treatment mean TEWL of 7.09 from a baseline mean of 26.6[1].

KCO is extracted from the fresh, mature kernel of the king coconut. It is colourless and has the aroma of fresh king coconut.

KCO has a high iodine value which indicates the presence of high amounts of unsaturated fatty acids in KCO. When the iodine value is high, the drying property of a particular oil will also be high. A drying oil hardens to form a tough solid film on exposure to air through a chemical reaction with oxygen in the air but not through the evaporation of water. Therefore, the drying property of oil is an advantage in producing cosmetic products because it improves the occlusive function of the epidermis. Comparatively higher levels of free fatty acids are found in KCO[22].

Liquid paraffin is a standard base in most of the moisturizers. It can also be used alone as a moisturizer. Liquid paraffin is a colourless, almost odourless, oily liquid composed of complex saturated hydrocarbons obtained from petroleum[23].

The appearance, colour and odour of VCO, KCO and liquid paraffin are so similar that they cannot be distinguished from each other. Therefore, we used the liquid paraffin as a comparator in our study.

Materials and methods

Study design

The pilot study was conducted as a part of a randomized, double-blind, parallel-group, comparison trial assessing the efficacy and safety of VCO and KCO compared with liquid paraffin as a moisturizer for mild AD. Ethical approval was taken from the Institutional Ethics Review Committee of Faculty of Medicine, University of Peradeniya (2020/EC/62) and the Clinical Trial registration number is SLCTR/2021/006. Written informed consent was obtained from all subjects and the study was conducted in accordance with the Declaration of Helsinki.

Preparation of coconut and king coconut oils

Preparation of VCO and KCO done at Coconut Research Institute Sri Lanka as follows,

Seasoned Coconut/ King Coconut
Dehusking
Deshelling
Paring
Splitting
Washing with potable water
Disintegrating
Drying (at less than 650C)
Feeding into cold pressing oil expeller (Cold Pressing Oil Expeller is an expeller that operates ~ 600C)
Extraction of virgin coconut oil/ virgin king coconut oil (~ 600C)
Raw virgin coconut oil/ Raw virgin king coconut oil
Sedimentation
Filtration or centrifugation
Storage
Bottling and sealing
Labelling
Storage of VCO and KCO

Liquid paraffin was purchased from the State pharmaceutical corporation Sri Lanka.

Participants

The study population was the patients with mild AD (SCORAD index of 0-24) aged more than two years. The following categories of patients were excluded from the study: patients with presence of AD only on the face, patients with significant mental and physical disabilities, pregnant or lactating women, patients who had used topical or oral steroids during past two weeks, patients who had used topical calcineurin inhibitors during past two weeks, patients who had used systemic immunosuppressive therapies (methotrexate, cyclosporine, azathioprine, mycophenolate mofetil) during past two weeks, patients with currently infected lesions that require antibiotics, patients with other dermatological conditions in addition to AD (e.g. Psoriasis) patients with known hypersensitivity to VCO, KCO or liquid paraffin, patients with any concomitant medication that could aggravate AD (retinoids, diuretics, lipid-lowering agents, calcium antagonists, beta blockers) patients with known comorbidities that could aggravate AD (heart failure, chronic kidney disease, diabetes mellitus, chronic liver diseases, hyperparathyroidism, hypo or hyperthyroidism, iron deficiency).

The patients were recruited from the outpatient dermatology clinic "Skin Center", Sirimavo Bandaranayake Mawatha, Kandy, Sri Lanka. The study was conducted at the Department of Pharmacology, Faculty of Medicine, University of Peradeniya as a single-center study.

Sample size

The study was conducted as a pilot study (phase III clinical trial) with three study arms with a total sample size of 35 (Table 2).

Table 2: Three study arms with a total sample size of 35

Study arm (moisturizer treatment for AD)	Sample size
VCO	11
KCO	12
Liquid paraffin	12

Randomization

Patients were assigned to the three treatment arms equally using a block randomization method.

Allocation concealment and blinding

VCO, KCO and liquid paraffin which are similar in colour, odour and consistency were packaged in identical bottles by the pharmacist and labelled as "A", "B" or "C". Only the pharmacist knew what type of oil was filled in each bottle labelled as "A", "B" or "C and the allocation sequence was kept secret in a sealed envelope until the end of the study. The co-investigator who did the randomization informed the pharmacist to dispense the relevant bottles of oil to the patient. The pharmacist recorded the letter assigned to each subject.

Intervention

Study interventions were the use of VCO and KCO as a moisturizer. A comparison intervention was the use of liquid paraffin as a moisturizer.

The trial was conducted for a total period of four weeks including a recruitment visit and two follow-up visits. Screening was done by the principal investigator (PI) using the Hanifin and Rajka criteria for diagnosis of AD and eligibility of the subjects was assessed and informed written consent was taken from eligible subjects.

Outcome measures

At the recruitment visit baseline severity of the disease was assessed clinically by using SCORAD index and POEM score and baseline instrumental measurements of skin moisture level and skin lipid level. SCORAD index ranges from 0 to 103 and POEM index ranges from 0 to 28. The lowest means the lower severity of the disease in both scores. At each follow-up visit done at 2 and 4 weeks, the severity of the disease was assessed as in baseline. Occurrence of adverse events and compliance were also assessed at each follow-up visit.

Statistical methods

A series of linear mixed-effects models were fitted to assess the outcome variables, including differences in SCORAD scores, POEM scores, lipid levels, and moisture levels between visits. The treatment arm was modelled as a random effect in these models, and they were compared to corresponding intercept-only fixed effect base models using ANOVA.

To compare SCORAD scores, POEM scores, lipid levels, and moisture levels across visits within each treatment arm, Wilcoxon signed-rank tests were conducted. A p-value less than 0.05 was considered statistically significant.

Results

Sixty-one patients were recruited and thirty-five patients between age three and 66y with mild atopic dermatitis completed the study. Eighteen (51%) of them were females whereas 17 (49%) were males. Patients were divided into three groups (X=12, Y=12, Z=11) and were given KCO, liquid paraffin and VCO respectively and were followed up for a total of three clinic visits. Baseline data are shown in Table 3.

Table 3: Baseline data of outcome measures

Arm	SCORAD (median of the first visit)(IQR)	POEM (median) (IQR)	Moisture level (median) (IQR)	Lipid level (median) (IQR)
X(KCO)	18.83 (23.02)	9.5 (7.75)	33(1.5)	2(1)
Y (liquid paraffin)	19.97 (10.83)	10.5 (7.5)	33(4.5)	2(2)
Z(VCO)	23.8 (19.5)	12 (8)	33(1.5)	2(0.5)

The comparison of the improvement of the SCORAD index, POEM score, moisture level and lipid level between the first and third visits using the Wilcoxon test which is a paired nonparametric test.

A significant improvement was shown in the SCORAD index of all three arms ($p < 0.01$). POEM score also showed a significant improvement in the disease in X (p=0.03), Y (p=0.01) and Z (p=0.01) arms. Significant elevation of moisture level was shown in the X (p=0.03) and Y (p=0.005) arms. No statistical significance was shown in the lipid level in all three arms (Table 4).

Table 4: The summary of the data at the end of the third visit

Arm	SCORAD	POEM	Moisture level	Lipid level
X(KCO)	P<0.01	P=0.03	P=0.03	Not significant
Y(liquid paraffin)	P<0.01	P=0.01	P=0.005	Not significant
Z(VCO)	P<0.01	P=0.01	Not significant	Not significant

The linear mixed-effects models, with treatment arms treated as random effects, did not demonstrate a significant improvement over the base models.

There was no statistically significant difference across different treatment arms in our previous analysis when compared with generalized linear models.

Regarding adverse events, one patient had itching when applying the liquid paraffin and another one had a burning sensation and erythema when applying KCO, however none has stopped the treatment due to adverse effects of the treatments.

Discussion

We were unable to achieve the intended sample size as most patients were reluctant to be treated only with a local application. There was about a 50% dropout rate. If the topical application could be combined with an oral tablet (placebo), the patients would have been more compliant with the trial. Therefore, we are planning to conduct a larger trial with oral placebo to achieve our main objective, which was to evaluate the efficacy and safety of VCO and KCO compared to liquid paraffin as a moisturizer for mild atopic dermatitis.

This is the first study of this nature comparing vegetable oils with hydrocarbon-based oils in Sri Lanka.

Another drawback of the study is not including the patients with facial eczema to the study. Most of the

patients with mild eczema have facial lesions. As we have not come across any adverse effects related to those three oils, next time perhaps we can include patients with facial eczema as well.

This study will be further extended on a larger scale to assess the ability of VCO and KCO to treat atopic dermatitis.

Conclusion

This pilot study shows that VCO, KCO and liquid paraffin are equally effective for the treatment of atopic dermatitis. As VCO and KCO are relatively inexpensive and widely available in Sri Lankan setup, this pilot study shows that they can be used instead of liquid paraffin in the treatment of mild atopic dermatitis in Sri Lanka.

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Effect of *Desmodium triflorum* on bleeding time: A pilot study

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Abstract

Natural herbs are popular in current society due to less side effects. *Desmodium triflorum,* is such a valuable plant and crushed leaves are applied on minor wounds to prevent bleeding by villagers. Anyway, the scientific investigations carried out on medicinal plants are fewer. Thus, the current study was launched in the Faculty of Indigenous Medicine to fill the gap to a certain extent and to find out the effect of *Desmodium* leaves on bleeding time. The students of the faculty were considered as the target group due to easy handling and narrow age limit. Bleeding time was tested with the less invasive Duke method. As the control, normal bleeding time of the selected group (n=16) was obtained. The same group was used for the test also, to overcome issues such as age gap, sex gap and health differences. In the test, the pricked site was applied with pure crushed leaves (25 mg) of *Desmodium.* Then, at every 30 seconds, the site was screened for bleeding with the blotting paper (after removing the plant materials). The bleeding time was obtained at the time of stoppage of bleeding. The average bleeding time of control and test was 71.50 and 39.50 seconds respectively and the difference was statically significant ($P<0.05$) under the paired t test. It is obvious that the bleeding time has an effect from the crushed plant materials. Bleeding time is controlled mainly by the constriction of blood vessels and formation of platelet plugs in the wound area. The reduction of bleeding time by the plant could be due to its phytochemicals such as tannin, flavonoids which have astringent effect that reduce the blood flow to the area. Further, Anti prostaglandin property of phytochemicals reduce vasodilation. Thus, the study will be a platform for scientists to conduct more investigations on the plant and develop a naval drug for wound bleeding.

Keywords: *Desmodium triflorum*, phytochemicals, astringent, anti-prostaglandin, bleeding time

Introduction

From ancient time, hand remedies have been used by villagers as emergency medicine in Sri Lanka. Among them *Desmodium triflorum* (*Heen Undupiyaliya*) has been commonly used in arresting fresh wound bleeding. Whole fresh plant is crushed and applied around the wound as a remedy to arrest bleeding. Though several such hand remedies are available, only a handful amount has been investigated scientifically to explore their effectiveness/mechanism of action. Thus, this *in-vitro* study was planned to find the effectiveness of *Desmodium triflorum* on bleeding. When a fresh wound is occurred, two body mechanisms come in to arrest bleeding. The damage vessels constrict and try to reduce the blood flow toward the site. Formation of platelet plug also seal the damaged vessel. These two mechanisms can manage minor bleeding. Thus, how fast small vessels in the skin stop bleeding[1] is called bleeding time and the normal reference range, is 2-7minutes[2]. The clotting cascade come into play later and stop bleeding completely by making a blood clot. In this *in-vitro* study, it is to test the effect of *Desmodium triflorum* on bleeding time, the Duke method was used here in the test to calculate bleeding time as it is less invasive, user friendly and commonly used in hematology. *Desmodium triflorum* which is in Fabaceae family is a small, perennial herb and leaves are small, alternate, stipulate and trifoliate.

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Stipules ovate in shape. Flowers are small, white or pink colored[3] (Figure 1).

Fig. 1: Plant *Desmodium triflorum*

In extracts of *D. triflorum*, it has revealed the presence of alkaloids, flavonoids, proteins, phytosterols, saponins and tannins[4.5].

As the researchers, there was a research problem to know whether there is a true effect by *Desmodium triflorun* on decreasing bleeding time. Thus, the objective of the current study is to find out the effect of *Desmodium triflorum* on bleeding time.

Materials and methods

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The ethical approval was obtained from the ethic review committee of the Institute where the study was carried out.

This study designed as an experimental study and conducted in Physiology Laboratory, Faculty of Indigenous Medicine (FIM), University of Colombo, Sri Lanka and the time period was October 2022 to January 2023.

The students of the FIM were made involve in the study, due to the facts that the university students are young, less of hematological diseases, supportive and easy to handle.

Participant were selected randomly on lottery base method. The participants were acknowledged first regarding the study and the information forms were given, then the consent was obtained. They were given equal chances to ask questions for their further knowledge. Investigators asked questions on the study to clarify that the participants understood about the research. The participants were informed that they would leave the study freely at any time if they would not wish to continue the research study.

Exclusive criteria

- The participants who had been on warfarin, Aspirin, NSAIDs, or Alcohol for last 7 days prior to the test
- Participants with hemophilia

Inclusive criteria

- Participants who were healthy and wished to participate in the study

Selection and preparation of plant extract

The selected plant was identified and authenticated with the Department of *Dravyaguna vignana* in the FIM. The total plant was cleaned, washed and air dried. It was crushed with a mortar and pestle.

Bleeding time test [2]

Both test and control were done on the same individual to avoid issues such un -machining of age, sex and health condition in the two groups.

Control experiment

Fingertip of the participant was sterilized well with surgical sprite (70% Ethyl alcohol) and the area was dried and puncture (non - deep, up to 3mm) with a disposable non expired Lancet. The wound was swabbed with filter paper every 30 seconds until no more blood was absorbed. The total time since the puncture to no more blood stain was seen in filter paper was considered as bleeding time. Normal reference rage of bleeding time for Duke method is 1-3 minutes[6].

Test experiment

Fingertip of the participant was sterilized well with surgical sprite (70% Ethyl alcohol). The area was dried and puncture (non – deep / up to 3mm) with a disposable non expired Lancet. The first blood drop of the wound was swabbed with filter paper and counting time started. Eventually, a small amount of crushed plant material (25 mg) was kept in the

puncture site. Then, after every 30 seconds, the crushed plant material was removed carefully, blood was wiped out with filter paper and replaced the material quickly. Thus, time was counted until no stain of blood was seen in the filter paper.

Statistical analysis

Paired t test of the SPSS statistical package was utilized to make a statistical comparison between control and test results of bleeding time.

Results and Discussion

In this pilot study, 16 students were tested and the distribution of male and female were 37% and 63%. The average age of the study group was 28 years. Under the sex distribution of the study group, the male was predominant.

The bleeding time of test group and control group is shown in Table 1.

Table 1: The bleeding time of test group and control group

Test	Average time value of the control group (n = 16)	Average time value of the test group (n = 16)	Statistical Significance
Bleeding time	71.50 seconds	39.50 seconds	P < 0.05

According to the result, the bleeding time was significantly reduced in the test group compared to the control group, and this result was statistically significant. Therefore, it is clear that the decrease in bleeding time in the control group is due to the effect of the crushed plant materials.

The arrest of bleeding is a result from a combination of four mechanisms: vasoconstriction, platelet embolism, platelet clot formation, and fibrinolysis. Previous research has found that *Desmodium triflorum* has several active components, such as, Alkoloids, Flavonoids, Saponins and Tannins[7,8]. Among them, Tanins are vaso protective, they limit the fluid loss and promote regeneration of tissues in case of superficial wounds or burns[9]. Tannins also precipitate proteins to form vascular plugs[10]. Flavonoids have an anti-hemorrhagic effect due to stabilization of capillary integrity[11], it triggers platelet aggregation also. Thus, it is obvious that phytochemicals in the plant help constrict the blood vessels to reduce bleeding. More overly phytochemicals inhibit the formation of prostaglandin, which occurs during vessel wall injury. Prostaglandin is responsible for vessel relaxation and increase in bleeding [12]. More overly, Saponin in *Desmodium* is effective in stimulating the production of collagen, which is important for wound healing [(13)]. When considering the previous research studies mentioned above, it is clear that the plant contains phytochemicals and its effect on reducing bleeding has occurred due to their assistance. Thus, this could be the reason for using this plant by villagers to arrest bleeding from minor wounds for a long time.

Conclusion

Desmodium triflorum crushed plant materials has an effect on the reduction of bleeding time of minor wounds. The results of this study will help researchers to do more studies on the topic and detect the phytochemicals acting as hemostatic agents which have astringent properties/ vasoconstriction effects to develop a drug for bleeding disorders. Moreover, the outcome of the study could be limited by the number of participants, as well as on the technique and chemicals which were used. The present study has been carried out with a limited number of participants such as 16. This could limit the outcome of the research. Thus, it's better to improve the research with a greater number of participants. Further, in the study Duke method has been used to detect the bleeding time. it's better to follow more than one method such as Ivy method and to justify the outcome of the study.

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Siddha and Ayurvedic management of Ovarian Cyst: A case study

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

Abstract

Ovarian cysts are common finding in general gynecology. An ovarian cyst is one of the most common causes of ovarian dysfunction, which has a direct impact on fertility. A female patient aged 18 visited the outpatient clinic of the Herbal Health Care Centre in Jaffna, Sri Lanka, on 19.01.2021, with lower abdominal pain for two years, reduced menstrual intervals for one year, and pain in the right lower quadrant. She reported having experienced localized soreness a few months back. There was a sudden onset of pain without any prior history. The left ovarian cyst (size-43 mm) in an ultrasound scan reported. The laparoscopic approach for removing the cyst. The woman had somewhat recovered from her ovarian cyst symptoms, nevertheless. Then the left ovary had a 14mm cyst, per the results of the ultrasound scan that was done on 2019. According to the ultrasound scan obtained on 2020, as depicted in, the cyst was gradually expanding and had grown to a diameter of 23 mm. At the patient's request, the doctors affiliated with the herbal health care centre began the Siddha Ayurvedic course of treatment given *Sathavary* (*Asparagus racemosus*) decoction 60 ml twice a day, before meal, *Karisalai* (*Eclipta alba*) syrup 20ml twice a day after meal. Tripala tablet 2, twice a day after meal. *Kanchanara guggulu* 2 twice a day after meal. The patient has seen a demonstration of *Kayakalpha* exercise. There are two exercises in it. *Aswini Mudra* and *Ojus Breath* are among them. On September 1, 2021, the ninth day of the most recent menstrual cycle, an ultrasound examination was carried out six months after the patient had begun treatment. According to the report, the left ovary was normal in size and appearance, and the ovarian cyst had shrunk in size. This case study serves as an example of how Siddha Ayurvedic medicine can effectively treat ovarian cysts without the need for surgery.

Keywords: ovarian cyst, Sri Lanka, *Karpa vaayu*, *Tridoshasa*

Introduction

Ovarian cysts are common finding in general gynecology. An ovarian cyst is one of the most common causes of ovarian dysfunction, which has a direct impact on fertility[1]. Ovarian cysts are ovarian follicles that are greater than two cm in diameter. Cyst in the ovary is closed sac formations filled with a liquid or semi -solid material[2]. Ovarian cysts can affect women of any age, although they are more common in women who are pregnant or planning to get pregnant. The majority of ovarian cysts are benign. Irregular periods, abnormal uterine bleeding, abdominal or pelvic pain, exhaustion, headaches and nausea are all common signs of an ovarian cyst.

Ultrasound, MRI and CT scans are used to detect ovarian cysts[3]. In today's medical system, an ovarian cyst is treated mostly with hormonal therapy (combined oral contraceptive tablets) or surgical therapy (pelvic laparoscopy)[4]. This is the only treatment for ovarian cysts available in modern medicine to meet the patient's urgent needs, and challenges remain to establish a satisfactory conservatory medical treatment to this day, the lack of conservative and satisfactory treatment in biomedicine necessarily requires the search for

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conservative and satisfactory treatment in another medical system.

Sinaippai neerkattigal, Karpa neerkattigal, Soolaga neerkattigal, and *Karpa vaayu* are Siddha terminology that can be connected with the symptoms of Ovarian cyst, according to traditional Siddha literature.

Danvanthri Vaithiyam and Agathiyar Amuthakalai Gnanam discuss the etiological factors of reproductive illnesses. According to this, eating high-calorie foods such as starchy foods, milk, and fruits during menstruation causes *Vatham* (*Vayu*) to build up in the uterine cavity and causes aberrant muscle growth in the uterus. This results in decreased blood flow to the organ, which causes amenorrhea, obesity, lower abdomen pain, and infertility[5]. When the *Vayu* remains trapped in the uterine cavity, *Pitham* also accumulates in the uterus, according to one of the oldest works of Siddha literature from the year 1500, Ganavertian. The regular menstrual cycle is obstructed as a result of this *Vatham* and *Pitham* accumulation. The body develops an abnormality that causes obesity. Blood stagnation causes abnormal *Vayu* production, as well as an accumulation of *Vayu* in the anal region[6].

Case History

A female patient aged 18 visited the outpatient clinic of the Herbal Health Care Centre in Jaffna, Sri Lanka, on 19.01.2021, with lower abdominal pain for two years, reduced menstrual intervals for one year, and pain in the right lower quadrant. She reported having experienced localized soreness a few months back. There was a sudden onset of pain without any prior history. The patient reported weight gain without any associated nausea or vomiting. There was no notable reproductive system history in the patient's family. Menarche occurred at the age of 13, as revealed by the patient's menstrual history. Vital and systemic signs were consistent, and the menstrual cycles were regular. The Vaginal examination revealed a normal-sized, anteverted, fornices-free, mobile, and uniform uterus.

On Examination

General condition – good

Family History – normal

Vitals Examination

Blood pressure - 120/70mmHg,

Pulse rate - 86/minute

Weight- 76kg and Height-163.5 cm, BMI-26 Kg/m2

Personal history

Appetite-Poor

Sleep- normal

Bowel-constipation

Bladder-clear

Blood Investigation

Hb-10.3 gm%, TLC -5300 /mm^3, ESR-22mm/hr, Neutrophill-43%, Lymphocytes-53%, Eosinophil -2%, Monocytes- 2%, Basophils -0%.

T_3- 1.22ng/dl, T_4 –12mcg/dl, TSH- 5.23 mcIU/ml, FSH- 4.65mIU/ml, LH- 12.75mIU/m (LH: FSH is >2:1).

Figure 1 depicts the left ovarian (size-43 mm) in an ultrasound scan performed by a VOG attached to the Teaching Hospital Jaffna on April 9, 2016, in accordance with the Past Medical and Surgical History. The patient mentioned above has since received hormone therapy and had a laparoscopy (done in 2017). The laparoscopic approach for removing the cyst. The woman had somewhat recovered from her ovarian cyst symptoms, nevertheless. Table 1 Shows the before and after the allopathic treatment and Table 2 shows the in the laparoscopy and after laparoscopy management.

Table 1: Before & after allopathic treatment

Before treatment	After treatment
Left ovarian cyst (size-43 mm)	Left ovarian cyst (size-45 mm)

Table 2: In the laparoscopy & after laparoscopy Management

In the Surgery	After Surgery 1st visit	After Surgery 2nd visit
Left ovarian cyst (size - Nil)	left ovarian cyst size -14mm	left ovarian cyst size -23 mm

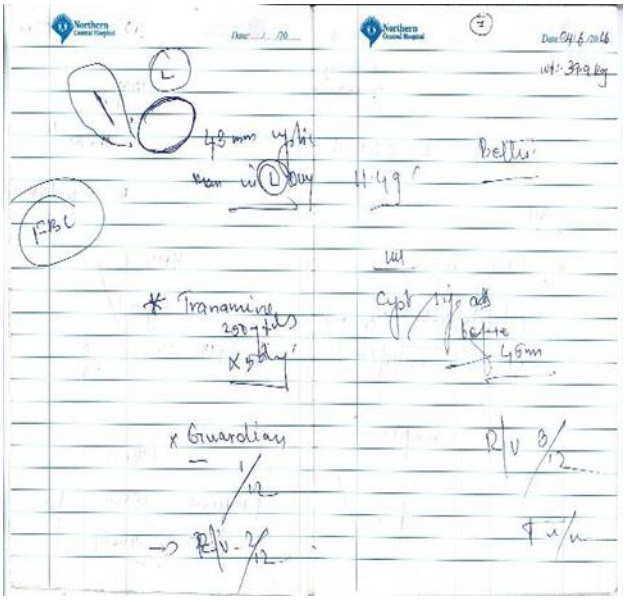

Fig.1: Ultrasound scan-09.04.2016

The left ovary had a 14mm cyst, per the results of the ultrasound scan that was done on April 29, 2019 (Figure 2). According to the ultrasound scan obtained on July 8, 2020, as depicted in Figure 4, the cyst was gradually expanding and had grown to a diameter of 23 mm.

Fig. 2: Ultrasound scan -29th April 2019

Fig. 3: Ultrasound scan - 08th July 2020

When she visited the Herbal Health Care Centre on, February 19, 2020, the regular physical and systemic examination were unremarkable, and the thyroid function test, liver function test, hormone profile, and ultrasound examination of the internal organs were all normal. At the patient's request, the doctors affiliated with the herbal health care center began the Siddha Ayurvedic course of treatment (Table 3). The initial medications were changed at 12th week and following medicines were prescribed (Table 4).

Table 3: Treatment plan (first twelve weeks)

Medicine	Dosage
Sathavary (*Asparagus racemosus*) decoction	60 ml bid, before meal for 8weeks
Karisalai (*Eclipta alba)* syrup	20ml bid, after meal for 12 weeks
Tripala tablet	2 tab. bid, after meal for 12 weeks
Kanchanara guggulu	2-tab, bid, after meal for 12 weeks

Table 4: Treatment plan (second twelve weeks)

Medicine	Dosage
Sathavary (*Asparagus racemosus*) decoction	60 ml bid, before meal for 12weeks
Katpakaddy (Uterine fibro reduction) decoction	60 ml bid after meal for 12 weeks
Katalai (Aloe vera*)* syrup	30 ml bid after meal for 12 weeks
Tripala tablet	2 tab. bid after meal for 12 weeks
Kanchanara guggulu	2-tab bid after meal for 12 weeks

The patient has seen a demonstration of *Kayakalpha* exercise. There are two exercises in it. *Aswini mudra* and *Ojus breath* are among them. The nervous system needs to be strengthened in order to withstand aging and delay death. *Aswini mudra* or nerve-toning exercise is what this is. The *Aswini mudra* strengthens and tones the neurological system. The body's internal organs are healthy enough to perform their functions. *Ojus breath* sublimates the joints and sexually active fluid at its source. The electrified life force and biomagnetism

are transferred from the *Mooladhara* to the brain cells and then dispersed throughout the bodily cells during the *Ojus breath.* The fluid of sexual vitality gets purer and denser. During this period the patient was advised to take balanced diet and nutritive diet Ghee, milk, fruits, green vegetables. Avoid oily, spicy, junk foods.

On September 1, 2021, the ninth day of the most recent menstrual cycle, an ultrasound examination was carried out six months after the patient had begun treatment. According to the report (Figure 4), the left ovary was normal in size and appearance, and the ovarian cyst had shrunk in size (Table 5).

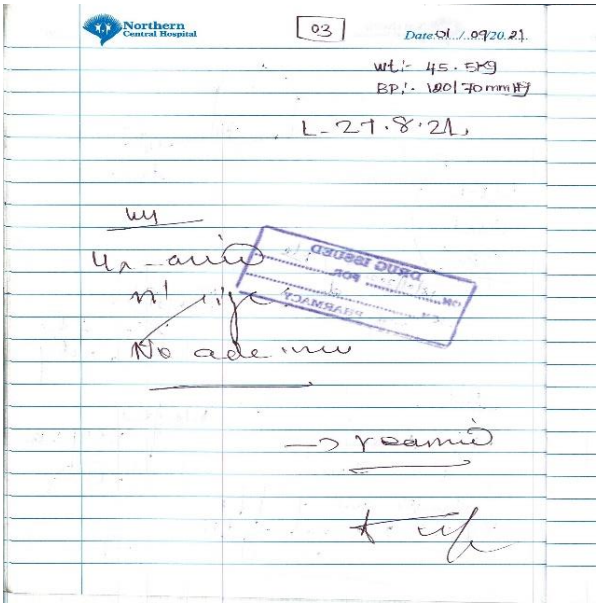

Fig. 4: Ultrasound scans -1st September 2021

Table 5: Before & after Siddha & Ayurvedic treatment

Before treatment	After treatment
Left Ovarian Cyst (size-23 mm)	Left Ovarian Cyst (size-Nil)

Discussion

In Siddha Medicine classics, exact correlation of ovarian cyst cannot be found, but can be included under the broad term of *Karpa Vaayu*. The extensive inflammatory alterations occur in ovarian cyst sufferers. Inflammation and excessive weight gain are related in Siddha and Ayurveda, where they are associated with *Sama vastha amavastha* toxins. Unhealthy diet and lifestyle lead to the creation of *Ama* in *Rasa thathu,* which results in *Arthavaupatha thuthusti*, according to *Apathyya ahara viharas*. The ovum is improperly selected and matured as a result of this vitiated state. The leftover *Thathus* is so vitiated by the *Aama*, which shows itself as severe weight gain and hair loss. Hair is the *Mala roopa* of *Asthi,* and *Asthi dhathu dushti* (bone tissue degeneration) causes undesirable hair growth and hair loss. When *Mamsahara* is consumed in excess combined with *Avyayama* and *Divaswapna* (day sleep), *Kapa* and *Medhothusti* occur. Drugs with actions like *Amapachana, Agni deepana* (Carminitive), *Pasana* (digestive), *Vathanulomana, Lekhana* (reducing), and *Artavajanana* (ovulation induction) should be used[7] to normalize this situation. *Sathavary* decoction balances the *Vata* and *Kapha doshas* and has *Deepana* (increases stomach fire), *Pachana* (aids in digesting), *Rochana* (stimulates appetite), and *Anulomana* (improves breathing) properties. In addition to their bioactive components, the *Sathavary* plant parts are a superb source of nutrients and minerals that are good for health, including manganese, copper, zinc, cobalt, potassium, selenium, calcium, and magnesium[8]. Along with vital fatty acids like gamma-linolenic acid, the plant also contains vitamins like Vitamin A and ascorbic acid, which are important for treating diseases including hypercholesteremia, depression, and diabetes[9]. *Shatavar*i is known as a potent herb that is good for women and helps with many hormonal issues. In addition to aiding in the treatment of endometriosis, which is an inflammation of the uterine lining, it also strengthens the female reproductive system and promotes the development of the eggs into follicles[10]. Being a powerful tonic of the uterus, using this formulation regularly or consuming foods that improve fertility can be very helpful. *Kanchanara guggulu* contains anti-inflammatory, *Lekhana* (scrapping), and *Vata-Kaphasamana* effects. The cytotoxic impact of *Kanchanara guggulu* inhibits cell division and lowers cell proliferation[11]. It is discovered that by enhancing digestion, it is useful in balancing *Kapha*. *Kanchanara* (*Bauhinia variegate*)'s anti-

inflammatory and anti-diabetic qualities[12] aid in lowering insulin resistance, which is frequently linked to PCOS. The *Thiripala* tablet shields the body from mutagenic, inflammatory, and free radical damage.

Additionally, the hypoglycemic action of this drug reduces insulin resistance. Due to the aforementioned characteristics, vitiated *Dosha* and *Jadaragni* (digestive fire) are fixed, *Srothoshodana* takes place, and *Doshas* are expelled from the body. *Kapha* and *Medhas* are reduced by *Lekhana* property. The characteristics of *Rasayana* and *Arthavajanka* bring the female reproductive system back to normal.

Conclusion

Ovarian cysts make up a large proportion of the gynecological problems that women deal with daily. Correct Siddha Ayurvedic treatment, along with food change, aids in the regression of the cyst and related symptoms. This case study serves as an example of how Siddha Ayurvedic medicine can effectively treat ovarian cysts without the need for surgery.

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Declaration of Conflicting Interests

Regarding the research, writing, and/or publication of this paper, the authors reported that they had no potential conflicts of interest.

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Comparative quality evaluation on branded *Triphala* tablets in Ayurveda drug market of Sri Lanka

Nallaperuma D.M.*, Silva H.H.S.S., Fernando H.D.R., Wijesekara P.M. and Jayasiri A.P.A.

Abstract

At present standardization is essential to guarantee the quality of products as the market of all commodities has become global. The objective of this study was to comparatively evaluate the quality of four different branded *Triphala* tablets and one capsule (sample A, B, C, D, and E) available in Ayurveda drug market of Sri Lanka. All the brands were purchased from Ayurveda drug market and assessed for organoleptic, physical, chemical and pharmaceutical parameters. According to the results, total ash values (5.4%, 5%, 3.3%, 5.7%, 4.9% respectively) were above the standard limit of 2%. Water-soluble ash (0.9%, 2.4%, 1.6%, 2.7%, 1.2%) and acid-insoluble ash (2.5%, 2%, 1.9%, 2%, 1%) values were <3%. Water-soluble extractive values (7.35%, 13.08%, 20.03%, 11.28%, 8.18%) were higher than alcohol-soluble extractive values (7.25%, 12.23%, 13.48%, 9.7%, 8.15%) in all brands. Loss on drying at 105^0C of all were below 12%. Ethanolic extracts of all were positive for tannins, flavonoids, phenols, steroids, glycosides and carbohydrates and negative for alkaloids, terpenoids and proteins. Only C was positive for saponins. TLC (Toluene: Ethyl-acetate: Formic acid/3:5:1) showed similar patterns for all brands. HPTLC fingerprints of all were alike in terms of number of peaks and their intensity, except tablet C with four additional peaks. A, C and E passed the weight variation test. Friability of A, B and C were below standard limit of 1%. Disintegration time of A was below the standard limit of 15minutes. Hardness ranged from 172N to 503N. As there is a considerable variation in physical and pharmaceutical parameters of all brands, it is urgent to maintain common standardization parameters in Ayurveda drug market.

Keywords: *Triphala,* Standardization, Physical parameters, Pharmaceutical parameters, HPTLC.

Introduction

In the current world, the market for various commodities has undergone a remarkable transformation, becoming increasingly interconnected on a global scale. This phenomenon holds true, particularly for health-related products, which are now being produced in various corners of the world with the aim of reaching markets across the globe. Amidst this global expansion, the importance of standardization holds a fundamental necessity. Standardization is the process of implementing and developing technical standards. It plays an important role in ensuring the uniformity of health-related products across diverse geographical regions, consequently guaranteeing consumers access to consistently high-quality products with well-defined constituents.

The leading organization in implementing global health standards is the World Health Organization (WHO). WHO, through its collaborative efforts and support, establish mechanisms for the integration of traditional plant medicines into primary healthcare programs. Since the global market for health-related products continues to grow day by day, the critical importance of standardization is a need of the world market. Proper standardization process must be continued from the initial point of raw materials and up to the final outcome of the finished products. The three main pillars of standardization are quality,

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safety and efficacy[1]. The assessment of safety and efficacy, ensuring the availability of an adequate supply of herbal medicines, while being mindful about adulteration and upholding standards for the quality control of both raw and processed materials is of vital necessity in the market of herbal products.

A conventional example of a traditional poly-herbal formula with a rich history of medicinal use is *Triphala*. This commonly used formula is composed of the pericarps of the fruits of *Terminalia chebula* Retz. and *Terminalia bellirica* (Gaertn.) Roxb. both of which belong to the Combretaceae family, along with *Phyllanthus emblica* Linn. from the Euphorbiaceae family in equal proportions, based on Ayurvedic Formulary of India (AFI). *Triphala* is honored for its numerous pharmacological actions, used in herbal medicinal practices. The pharmacological actions of *Triphala* span a diverse spectrum of health benefits, including its role as a potent laxative, capacity for rejuvenating ocular health[2], potential as an anti-diabetic agent[3], anti-inflammatory properties[4], function as an appetizer[5], anti-oxidant and anti-bacterial properties[6,7]. Each of these properties showcases the holistic nature of *Triphala*, reflecting its significance in preventive and curative aspects within the traditional medicine system.

Triphala, a prominent herbal drug widely employed in traditional medicinal practices within Sri Lanka, served as the focal point of this study. The primary aim of this study was to conduct a comparative evaluation of the quality attributes of five distinct commercial brands of *Triphala* formulations, denoted as A, B, C, D in tablet form, and E in capsule form, all of which are readily available within the Sri Lankan Ayurveda drug market. The selection of these five specific brands was based on their prevalence and common usage among Ayurveda medical practitioners in Sri Lanka. Subsequently, samples of all five brands were purchased from local Ayurveda drug market. The evaluation process consisted of a thorough analysis of the products, covering a range of parameters including organoleptic parameters, physical parameters, chemical parameters and pharmaceutical parameters[8].

Materials and methods

Three samples from each of the 5 brands of *Triphala* were purchased from local Ayurveda drug market to assess for their organoleptic parameters, physical parameters, chemical parameters and pharmaceutical parameters. Tablet forms were named as sample A, B, C & D and the capsule form was named as sample E. Basic information of all the 5 brands are shown in the Table no 1.

Organoleptic Evaluation

Color, odor, taste and texture were assed under organoleptic parameters. All the samples were examined under diffuse daylight to observe the color. A small portion of samples were placed on a dish and slowly and repeatedly inhaled the air of material to sense the odor. Pieces of samples were chewed and tasted for taste sensation. Samples were touched to detect the texture.

Physical Evaluation

Physical parameters such as, ash values (Total ash, Water-soluble ash & Acid-insoluble ash), extractive values (Water-soluble and Alcohol-soluble) and loss on drying at 105^0C were done in triplicate for each brand and the average was taken. Standard methods prescribed in WHO guidelines for quality control methods for medicinal plant materials were followed for each test[9].

Total ash

Crucibles with 4g of powdered samples were ignited in a muffle furnace at 550^0C for 5-6 hours till total white ash was obtained. The total ash values were calculated with reference to the air-dried powdered drug material.

Water-soluble and Acid-insoluble ash

To the crucibles containing residue after the determination of total ash, 25ml of 2M HCl was added and boiled gently for 5 minutes using bunsen burner. Then the solutions were filtered separately using Whatman filter paper no. 42 and the insoluble matter was collected on to it. Insoluble matter

retained on the filter papers was washed with hot water and then the filter papers were transferred to the same silica crucibles and ignited to constant weight in the muffle furnace at a temperature not exceeding 450^0C. The percentage of acid-insoluble ash values were calculated with reference to the air-dried powdered drug material. The above procedure was performed with 25 ml of distilled water to find the water-soluble ash value.

Water-soluble and Alcohol-soluble extractive values

Four grams of powdered samples were transferred to conical flasks with 100ml of water. The flasks were kept in the shaker for 6 hours and then they were allowed to stand still for 18 hours. The mixtures were filtered using Whatman filter paper no 1, 25ml of the filtrates were measured from each mixture and were transferred to porcelain dishes which the weights were previously measured. The dishes were placed on a water bath and the solvent was evaporated completely. After that they were dried in a hot air oven at 105^0C for 6 hours, cooled and finally weighed. The percentage of water-soluble extractive values were calculated with reference to the air-dried powdered drug material. The above procedure was performed with 100ml of ethanol to find the alcohol-soluble extractive value.

Loss on drying at 105^0C

Two grams of powdered samples were heated in a hot air oven at 105^0C till constant weight was obtained. The percentage moisture contents of the samples were calculated with reference to the air-dried powdered drug material.

Chemical Evaluation

Preparation of ethanolic extracts of the samples

Soxhlet extraction at 60^0C using 5g of sample powder from each brand and 250ml of 99% absolute ethanol was followed to obtain the ethanolic extracts of each sample. Ethanol in each extract was evaporated using rotary evaporator at 45^0C leaving a small yield of the concentrated extracts. The extracts were stored in air tight glass vials at 4^0C until taken for further analysis.

Phytochemical Screening[10]

Alkaloids, Tannins, Phenols, Saponins, Flavonoids, Terpenoids, Steroids, Cardiac Glycosides, Carbohydrates and Proteins were analyzed qualitatively in the five ethanolic extracts. The procedures followed for each phytochemical are shown in the Table no 2.

Thin Layer Chromatography and High-Performance Thin Layer Chromatography

Extracts were spotted on a pre-coated silica gel 60G F_{254} aluminum plate separately. Solvent system of Toluene: Ethyl acetate: Formic acid (3:5:1 v/v%) was used to obtain a clear separation of compounds. Developed TLC plate was visualized under UV radiation of 254 nm and 366 nm wave length. The plate was scanned with the HPTLC scanner using winCATS software.

Pharmaceutical Evaluation[11]

Weight variation

From each brand 20 tablets/capsules were weighed at random and average weight of the tablets/capsule were calculated. Then the individual weight of a tablet/capsule was compared with average weight.

Weight variation % = (Individual weight – Average weight)/ (Average weight) × 100%

(Acceptance criteria – Not more than 2 individual tablet weights deviate from the average weight by more than the deviation stated in the Indian Pharmacopeia/IP).

Friability

20 tablets from each brand were weighed and placed them in the rotating drum of the friability apparatus. The drum was rotated 100 times. The samples were reweighed and calculated the weight loss.

$$\text{Friability} = \frac{\text{Initial weight} - \text{Weight loss}}{\text{Initial weight}} \times 100\%$$

Table 1: Basic information of *Triphala* tablets and capsule

Brand	Manufacturing Date	Expiry Date	Batch No.	Dosage form
A	18.02.2023	18.02.2025	241	Tablet
B	05.06.2023	05.06.2024	039121	Tablet
C	04.04.2023	04.04.2024	252	Tablet
D	22.02.2023	21.02.2025	2517	Tablet
E	13.02.2023	12.02.2025	736A	Capsule

Table 2: Phytochemical analysis of ethanolic extracts of different brands of *Triphala* tablets

Phytochemical	Procedure	Observation
Alkaloid	Mayer's reagent test: 2 drops of the reagent was added to 2ml of each extract and mixed well. Wagner reagent test: 2 drops of the reagent was added to 2ml of each extract and mixed well.	Cream color precipitate Reddish color precipitate Reddish colour brown precipitate
Tannins	$FeCl_3$ Test: 5 drops of $Fecl_3$ were added to each extract and mixed well.	Black precipitate
Phenols	Lead acetate test: 3 drops of lead acetate solution was added to 5ml of each extract and mixed well.	Yellow precipitate
Saponins	Foam test: 5ml of each extract was mixed with 2.5ml of distilled water separately, shaken vigorously, and kept for 10 minutes.	Stable foam of honey comb appearance
Flavonoids	Ammonia test: 5ml of dil. Ammonia solution was added to 5ml of each extract followed by the addition of con. H_2SO_4.	Yellow color
Terpenoids	Salkowski test: 5ml of each extract was mixed with 2ml of Chloroform and 3ml of con. H_2SO_4 was added along the sides of the test tube.	Reddish brown color
Steroids	Lieberman Burchard test: 2ml of Acetic anhydride and 2ml of con.H_2SO_4 were added to 2ml of each extract and mixed well.	Dark bluish green color
Cardiac glycosides	Keller Kiliani's test: 1ml of Glacial acetic acid was added to 3ml of each extract and con.H_2SO_4 was introduced to the bottom of the tube.	Reddish brown ring at the interface of the two liquids
Carbohydrates	Benedict's test: 2ml of each extract was mixed with 3ml of Benedict's reagent and boiled for 2 minutes.	Brick red precipitate
Proteins	Biuret test: 2ml of each extract was mixed with 2ml of 1% NaOH and few drops of $CuSO_4$.	Purple color

Table 3: Weight variation limits of tablets as per Indian Pharmacopeia

Average weight of a tablet	% of weight variation acceptable
80mg or less	± 10%
80 – 250mg	± 7.5%
> 250mg	± 5%

Disintegration

One tablet was placed in each tube of the Disintegration apparatus and the basket of the apparatus was filled with distilled water at 37^0C. Then the time taken for all the tablets to disintegrate and pass through the mesh were observed. If any residue remains it must have a soft mass with no palpably firm core.

Hardness

Hardness of one tablet from each brand was tested using hardness tester.

Results

Organoleptic Evaluation

Brand A was having dark yellow color, brand B and C were having dark brownish green, brand D was having a dark yellow and brand E was having a light green color. Each brand was having a characteristic odor specific to them. Brands A, C and D were having sour taste and brands B and E were having a bitter taste. Brands A to E were having smooth texture.

Table 4: Results for the physical evaluation of different brands of *Triphala* tablets

Physical Parameters	Brand A M±SD	Brand B M±SD	Brand C M±SD	Brand D M±SD	Brand E M±SD
Total ash	5.4±0.10	5.0±0.10	3.3±0.10	5.7±0.10	4.9±0.10
Water-soluble ash	0.9±0.26	2.4±0.30	1.6±0.20	2.7±0.36	1.2±0.26
Acid-insoluble ash	2.5±0.20	2±0.20	1.9±0.10	2±0.20	1±0.20
Water-soluble extractive value	7.35±0.52	13.08±0.21	20.03±0.19	11.28±0.30	8.18±0.25
Alcohol-soluble extractive value	7.25±0.52	12.23±0.15	13.48±0.14	9.7±0.20	8.15±0.24
Loss on drying at 105^0C	8.6±0.10	5.15±0.20	7.75±0.13	7.1±0.30	9.1±0.10

Table 5: Results for the phytochemical analysis of ethanolic extracts of different brands of *Triphala* tablets

Phytochemical	Brand A	Brand B	Brand C	Brand D	Brand E
Alkaloid	-	-	-	-	-
Tannins	+	+	+	+	+
Phenols	+	+	+	+	+
Saponins	-	-	+	-	-
Flavonoids	+	+	+	+	+
Terpenoids	-	-	-	-	-
Steroids	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	-	-	-	-	-

Physical Evaluation

The Table 4 shows the results for the physical evaluation of different brands of *Triphala* tablets.

Chemical Evaluation

The Table 5 shows the results for the phytochemical analysis of ethanolic extracts of different brands of *Triphala* tablets. The figure 1 show the Thin Layer Chromatogram of ethanolic extracts under 256nm and 366nm UV light while the figure 2 shows the HPTLC fingerprint profiles of the ethanolic extracts of *Triphala.*

Pharmaceutical Evaluation

Table no 6 shows results for the pharmaceutical evaluation of different brands of *Triphala* tablets.

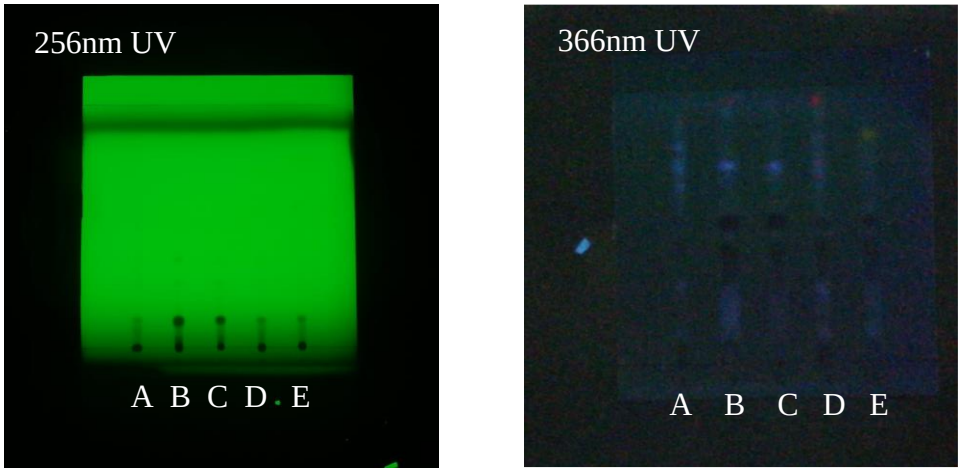

Fig. 1: TLC fingerprint profiles of the five ethanolic extracts of *Triphala* under 256nm and 366nm UV light

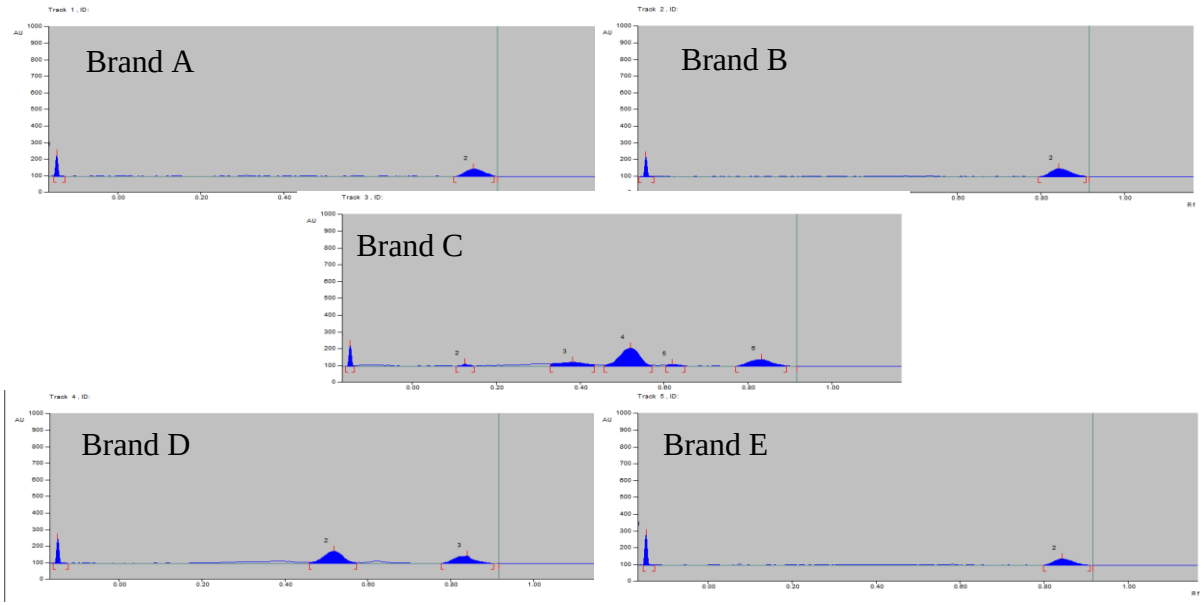

Fig. 2: HPTLC fingerprint profiles of the five ethanolic extracts of *Triphala*

Table 6: Results for the pharmaceutical evaluation of different brands of *Triphala* tablets

Pharmaceutical parameter	Brand A	Brand B	Brand C	Brand D	Brand E
Weight variation	Passed	Failed	Passed	Failed	Passed
Friability	0.03%	0.09%	00%	2.5%	--
Disintegration	12min	57min	30min	45min	--
Hardness	176N	205N	503N	172N	--

Discussion

Organoleptic studies showed more similarity in all the brands. Ash values are used to determine the quality and purity of a drug. Low ash values indicate more purity and quality while high ash values indicate contamination, substitution or adulteration of a drug. In this study, total ash values of all the brands were above the standard limit of 2%. These values were comparable with values obtained in a previous study on three different brands of *Triphala* tablets[12]. Acid-insoluble ash value indicates the presence of siliceous impurities while water-soluble ash value indicates the presence of inorganic contents. In this study those values for all the brand were below 3% which are within the standard limit[12]. These values were not comparable with the previous study[12]. Water-soluble extractive values are higher than alcohol-soluble extractive values in all brands in this study. The same result was obtained in the previous study[12]. Brand C was having the highest water-soluble and alcohol-soluble extractive values in this study. It indicates that most of the phytoconstituents in the samples were more extracted and soluble in water than alcohol. Loss on drying is used to determine the volatile components in a drug. Low moisture content prevent microbial growth and it is important for the stability of an herbal drug. In this study, loss on drying at 105^0C of all the brands were below the standard limit of 12% w/w% and these values were comparable with the previous study[12]. However, brand E was having a high loss on drying value when compared with the others.

In a previous study on *Triphala* tablets, the phytochemical screening of the aqueous extract of three different brands revealed the presence of tannins, flavonoids, phenols, steroids, carbohydrates, saponins while glycosides were present only in two brands[12]. In this study, ethanolic extracts of all the brands were positive for tannins, flavonoids, phenols, steroids, glycosides and carbohydrates and only extract C was positive for saponins. Ethanolic extracts of all the brands were negative for alkaloids, terpenoids and proteins. Difference in phytochemical screening may be due to collection of raw materials from different geographical locations, variations in quantity of each raw material, variations in excipients added in the production process, adulteration or substitution. Thin Layer Chromatogram showed similar patterns for all the brands. HPTLC fingerprints of brands A, B and E were similar in terms of number of peaks and their intensity. HPTLC fingerprint of tablet C showed four additional peaks and tablet D showed one additional peak.

In the pharmaceutical analysis, weight variation test determines uniformity in accordance with the formulation of each batch of tablets. In this study, tablets A, C and capsule E passed the weight variation test as per IP standards. Tablets B and D failed the weight variation test as per IP standards. Friability test determines the tendency of a tablet to chip or break upon compression. Friability of all the tablets in the study except tablet D were within the standard limit of 1%. Disintegration time determines when a tablet will disintegrate to reach dissolution. Tablet A had a disintegration time within the standard limit (<15min) while Tablets B, C and D exceeded the standard time for disintegration in this study. Hardness of a tablet determines the force required to break a tablet. Tablet requires a certain strength to withstand mechanical shocks on handling in manufacturing, packaging and transporting. In this study hardness of tablets of the different brands ranged from 172N to 503N.

Conclusion

Organoleptic and chemical parameters of all the brands of *Triphala* tablets had no considerable difference in their values. But physical and pharmaceutical parameters of all the brands of *Triphala* had considerable difference. The results of the study concludes that there is no uniformity in all the formulations. Therefore, there is an urgent need to make more rigid quality control parameters to maintain the standardization of *Triphala* tablets in the Ayurveda drug market.

Conflict of Interest

Not declared.

Acknowledgement

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Physico-chemical analysis of *Valu kashaya* (decoction prepared by using sand) used in *Suthika roga* (Complication of post-partum)

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Abstract

Valu decoction is a classical formulation mentioned in authentic Traditional textbooks. It is predominantly categorized into two types, namely *Valu* decoction I and *Valu* decoction II. While the method of preparation remains consistent for both methods, the ingredients employed differ. The decoction is primarily utilized to address post-partum complications. It consists of twelve plant-based ingredients and sand of river or stream. The present study primarily focuses to lay down analytical standards for *Valu* decoction I. The selection of quality and correct ingredients was the first step in standardizing herbal decoction. The relevant plant species were identified, and their validity confirmed using phytochemical investigation. The study was conducted to reveal the variations in organoleptic parameters (color, odor, and taste), physico-chemical parameters (pH, specific gravity, refractive index and brix value). Refractive index evaluates purity of preparation was found to be 1.33. pH value which evaluates the quality of the drug was found to be 5.18. Alkaline pH favors high microbial contamination of the herbal preparations, but it is slightly acidic in nature. Brix value use for evaluation of how much dissolved sugar is in a liquid solution was found to be 1.23. Foaming ability index was used to determine the foaming ability of aqueous decoction of herbal material was found to be1.36. Specific gravity is an evaluation parameter affirming wt/ml should always be more than carrier solvent (water). Notably, the majority of phytochemical constituents, including saponins, alkaloids, flavonoids, tannins, and steroids, were present in significant quantities across the samples.

Keywords: *Valu* decoction I, *Sutika roga*, Physico-Chemical

Introduction

Valu decoction is an herbal decoction used in Sri Lankan Traditional medicine for *Suthika roga* (Complication of post-partum). The term *Suthikawa*; refers to the mother until three months following childbirth. Various authentic texts elucidate distinct phases or time frames of the postpartum period, commonly known as *Suthika kala.* Susrutha/ Vagbhata mentioned *Suthika kala* as 11 – 01½ months, Kashyapa mentioned as 06 months and Bhava prakashaya mentioned as 04 months.

Suthika roga which refers to “postpartum disorders”, is widely discussed in Acharya Kashyapa, *Suthika Upakramaniya Adhyaya,* specifically within the *Kheelasthanaya* section, where he presents 64 distinct *Suthika roga*. *Suthika roga* is developed due to improper regimen, diet and mode of life, aggravated dosha, incompatible food, and digestive disturbances. Common signs and symptoms are body aches, fever, and tremors. The *Valu* decoction I mentioned in Pharmacopoeia consists of 12 plant-based ingredients and sand of river, distinguishing it from other decoctions documented in the Pharmacopoeia. However, when considering the historical context characterized by limited childbirth facilities, it is crucial to evaluate the efficacy of herbal decoctions in treating postpartum ailments such as wages *Sanniya* (puerperal fever) and *Suthika unmadaya* (postpartum-psychosis). In ancient times, practitioners used sand in the decoction after heating

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it until red-hot. In this regard, Pharmacopoeia highlights that due to a chemical component in the burnt sand, the filaments are poisoned. Further, it highlights the significance of the addition of crushed ingredients in the decoction process[1]. Table 1 mentioned the ingredients of *Valu* decoction I in Ayurveda pharmacopeia 1[2].

Table 1: Ingredients of *Valu* decoction I mentioned in Ayurveda pharmacopeia 1

Name	Sanskrit Name	Botanical Name	Used Part
Sudulunu	*Lasuna*	*Santalum album*	Bulb
Asamodagam	*Ajamoda*	*Trachyspermum ammi*	Seed
Kaluduru	*Kalajajii*	*Nigella sativa*	Seed
Thippili	*Magadhi*	*Piper longum*	Fruit
Inguru	*Shunti*	*Zingiber officinale*	Rhizome
Hathawariya	*Shathawari*	*Asparagus recemosus*	Root
Aralu	*Harithaki*	*Terminalia chebula*	Pericarp of the fruit
Iramusu	*Shariba*	*Hemidesmus indicus*	Root
Rasakinda	*Guduchi*	*Tinospora cordifolia*	Stem
Gammiris	*Maricha*	*Piper nigrum*	Fruit
Suduru	*Jeeraka*	*Cuminum cyminum*	Seed
Sassanda	*Ishvari*	*Aristoolochia indica*	Root

The identification and acquisition of the ingredients for *Valu* decoction II posed significant challenges due to their limited accessibility and the paucity of available references. The incorporation of references in research is essential for establishing a solid foundation to assess the quality, safety, and efficacy of drugs. Consequently, in light of the aforementioned limitations, *Valu* decoction I was selected as the subject of investigation for this research, as it offered a more viable opportunity to utilize existing references and comprehensively evaluate its medicinal characteristics[3].

Importance of standardization for Traditional decoction

Standardization helps to verify the identity, purity, and strength of components. It also facilitates scientific research on traditional decoctions, enabling accurate study of effects and correlations between formulations and therapeutic outcomes.

In the present study, The *Valu* decoction I was standardized by detection of physical parameters and chemical parameters, Screening of phytochemicals and developing of TLC profile.

Materials and methods

Collection and authentication of drugs

Raw materials were collected from local market at Embilipitiya and sand was collected from river – "Gin" in Galle.

They were authenticated by the Department of Ayurveda Pharmacology, Pharmaceutics and Community Medicine, Faculty of Indigenous Medicine, University of Colombo.

Preparation of Valu decoction I

There are two methods showing in Thalpathe piliyam[4], Deshiya Aushada Samgrahaya[5] and Ayurveda Pharmacopoeia[2]. However, considering the challenges encountered in sourcing certain ingredients and the constraints imposed by limited time availability, it was decided to proceed with method I as outlined in Ayurveda Pharmacopoeia part I.

First, the ingredients were crushed and subsequently bundled together. Next, a volume of water equivalent to 8 *Patha* (1920 ml) was added, and the mixture was placed within a vessel called "*Pottani*." Following this, clean and desiccated sand was heated until it attained a red-hot state. The vessel was then sealed using a hollow coconut shell, and the red-hot sand was added through the hole. This process facilitated the gradual reduction of the volume to 1 *Patha* (240 ml)[2].

Then did physico-chemical analysis of *Valu* decoction I according to WHO guidelines[6].

Physico-chemical parameters related to decoction

Physical evaluation

As the Physical parameters, pH value, forming index, specific gravity, refractive index and brix value are evaluated.

Chemical evaluation

As the Chemical parameters, qualitative phytochemical analysis, chromatography – TLC fingerprint profile for *Valu* decoction I has done.

Organoleptic properties

As the Organoleptic parameters, taste, odor, color were identified[7].

Quantitative analysis was conducted at equipped laboratory of Bandaranayake Memorial Ayurvedic Research Institute, Navinna.

Physical evaluations were done three times in each decoction and calculated the average on three samples, named S_1, S_2, and S_3.

Determination of the pH value

pH meter was used to measure the pH of the decoction at the 30^0C

Determination Brix value

Brix refractometer was used to measure the brix value.

Determination Refractive index

Determination refractive index was used to refractive meter.

Determination of specific gravity

The specific gravity of liquid is the relative weight of that decoction compared to an equal volume of water.

Determination of Foaming index

The foaming ability of an aqueous decoction of plant materials and their extracts were measured in terms of a foaming index[6].

Phytochemical screening of Valu decoction I

Freshly prepared extracts of *Valu* decoction I was subjected to detect the presence of phytochemicals[8].

Test for Tannins

Ferric Chloride Test - 5 drops of $FeCl_3$ were added 5ml of water extract of decoction and mixed well. Appearance of a black precipitate indicates the presence of Tannins.

Test for Flavonoids

Lead Acetate Test

Add few drops of 10% Lead Acetate to the 5ml water extraction of decoction. The appearance of a yellow color perception indicates the presence of Flavonoids.

Test for Alkaloids

Wagner's Test

To 5ml of water extraction of decoction add the 1% of HCL and Wagner's region. The appearance of a reddish-brown precipitation indicates the presence of Alkaloids.

Test for Saponins

Foam Test

Mix 5ml of decoction with distilled water and shake vigorously. Identification of positive result formation of stable more than 1mm.

Test for Steroid Glycosides

Equal volumes acetic anhydride and $CHCL_3$ were dissolved. The mixture was transferred to a dry test tube and con. H_2SO_4 acid was introduced to bottom of the tube. Formation of a reddish brown or violet-brown ring at the interface of the two liquids indicates presence of Steroid[8].

Development of Thin Layer Chromatography (TLC)

Valu decoction I was extracted in to Ethyl Acetate, concentrated and spotted on a pre-coated TLC plate. TLC fingerprint profile was developed using Toluene: Ethyl acetate: Methanol in a ratio of 6:2.5:1.5 v/v. The plate was visualized under UV radiation (both 254nm and 366nm).

Results

Organoleptic properties of prepared *Valu* decoction I is shown in Table 2.

Table 2: Organoleptic properties of prepared *Valu* decoction I

Taste	Astringent Taste (*Kashaya rasa*)
Color	Red-brown color
Odor	Mixed herbal odor

Physico-chemical of prepared *Valu* decoction I is shown in Table 3.

Table 3: Physico-chemical of prepared *Valu* decoction I

Test	S_1	S_2	S_3	(Mean Value)
pH value	5.24	5.12	5.2	5.18
Refractive index	1.33	1.33	1.33	1.33
Brix value	1.1	1.3	1.3	1.23
Foaming index	1.4	1.4	1.3	1.36
Specific gravity	0.8	0.89	0.8	0.83

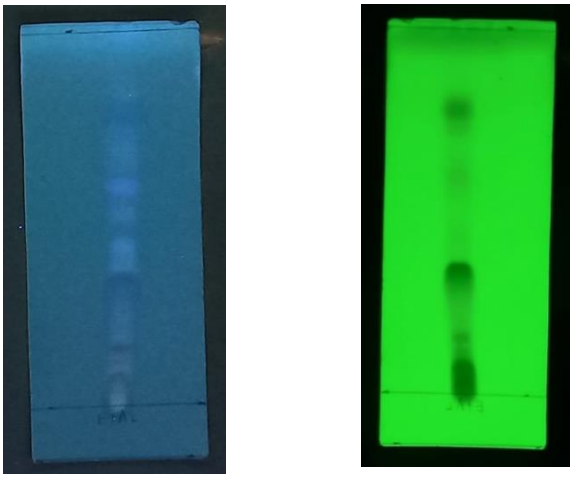
Table 4 shows the results of phytochemical analysis of *Valu* decoction I

Table 4: Phytochemical analysis of *Valu* decoction I

Phytochemical parameter	Results
Tannins	+
Flavonoids	+
Steroid glycosides	+
Alkaloids	+
Saponins	+

Figure 1 shows the TLC Fingerprint profile for prepared *Valu* decoction I

Sample: Ethyl - Acetate extract of *Valu* decoction I
Solvent system - Toluene:Ethyl Acetate: Methanol in a ratio of 6:2.5:1.5.
Visualization - (Under U.V Radiation)
Short U.V. 254 nm
Long U.V 366nm



At UV 366nm At UV 254nm

Figure 1: TLC photo documentation of ethyl alcohol extract of *Valu* decoction I

Discussion

The current preliminary investigation was undertaken to generate data on physic-chemical properties, including organoleptic characters, and chromatographic profiles to determine the quality and purity of *Valu* decoction I. The standardization parameters of liquid oral formulations such as refractive index, brix value, pH value, specific gravity, organoleptic properties were assessed to confirm flow property of formulation. Refractive index evaluates purity of preparation was found to be 1.33. pH value which evaluates the quality of the drug was found to be 5.18. Alkaline pH favors high microbial contamination of the herbal preparations, but it is slightly acidic in nature. Brix value use for evaluation of how much dissolved sugar is in a liquid solution was found to be 1.23. Foaming ability index was used to determine the foaming ability of aqueous decoction of herbal material was found to be1.36. Specific gravity is an evaluation parameter affirming wt/ml should always be more than carrier solvent (water). In the present study, it was found to be 0.83. The results of all three samples exhibited substantial similarity due to the use of correct raw materials in all three decoctions including identical temperature and location. The observed values remained consistent throughout the study. Notably, the majority of phytochemical

constituents, including saponins, alkaloids, flavonoids, tannins, and steroids, were present in significant quantities across the samples. *Valu* Decoction I was found to contain glycosides, which were detected through phytochemical screening tests. Most common postpartum complications are cardiovascular diseases, infection or sepsis, excessive bleeding after giving birth (hemorrhage), thrombotic pulmonary embolism, stroke, high blood pressure, anesthesia and anesthesia complications. Saponins, a major component in the formulation, play a significant role in the formation of immune-stimulating complexes and exhibit anti-inflammatory properties[4]. The primary pharmacological action of saponins is the reduction of blood lipids[5]. Alkaloids, on the other hand, possess anesthetic, cardio protective and anti-inflammatory properties. Flavonoids are known for their anticancer, antioxidant, anti-inflammatory, and antiviral properties. Tannins exhibit antioxidant, antimicrobial, and anti-inflammatory characteristics. The presence of these phytochemicals collectively contributes to the mitigation of postpartum complications.

Furthermore, Garlic (*Allium sativum*) possesses anti-inflammatory, analgesic, anti-stress and wound healing effects. *Suduru (Cuminum cyminum*) stimulates the digestive fire and promotes digestion, helping treating vomiting and diarrhea. *Sassanda (Aristoolochia indica)* helps in strengthening the uterus and promoting the overall well-being of women after childbirth. Ginger (*Zingiber officinale*) is commonly used to boost digestive power, hypertension, improve immunity and reduces pain. *Aralu* (*Terminalia chebula*) is used for its efficacy in managing diarrhea and dysentery, in addition to its anti-oxidant, antimicrobial, anti-inflammatory and cardio protective properties. *Kaluduru (Nigella sativa)* use for diarrhea and inflammatory conditions. Therefore, all these ingredients contribute to reducing postpartum complication.

Present study presents a preliminary attempt on development of a standardized manufacturing procedure for *Valu* decoction I. Further investigations can be conducted to assess the microbial composition through microbial testing and analyze the presence of heavy metals through heavy metal analysis. Moreover, additional research can be conducted to explore the therapeutic efficacy of *Valu* decoction I, as well as the two formulations of *Valu* decoction II. These subsequent studies would contribute to a more comprehensive understanding of the potential benefits of decoction and support its evidence-based application in therapeutic contexts.

Conclusion

Valu decoction I, as documented in Ayurveda Pharmacopoeia Volume 1, was prepared three times, using three distinct samples. Each of these samples was further divided into three sub-samples to facilitate the development of a standardized manufacturing procedure for *Valu* decoction I. It is important to note that the analytical values of *Valu* decoction I have not been specified in any authoritative literature. Therefore, these parameters can be taken as the preliminary standards for the further studies pertaining to *Valu* decoction I.

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Assessment of anti-oxidant activity and High-Performance Thin-Layer Chromatography characters of different compositions of *Triphala* powder

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Abstract

Triphala: a polyherbal formulation used in both Ayurveda and Sri Lankan Traditional Medical practices. Aim of the present study is to compare the chemical characteristics of distinct compositions of Triphala powder as mentioned in various Ayurveda Authentic Texts. Dried fruits of *Terminalia chebula* Retz. (TC), *Terminalia bellirica* Gaertn. (TB), and *Phyllanthes emblica* (PE) were purchased and authenticated. Followed by a comprehensive literature review, five different compositions of Triphala dried powdered samples as S_1 (1:1:1), S_2 (1:2:3), S_3 (1:2:4), S_4 (1:2:2) TC: TB: PE respectively and S_5 – 1:2:4 ratio based on the fruits of TC: TB: PE were subjected to ethanol extractions. Anti-oxidant activity and High-Performance Thin-Layer Chromatography (HPTLC) characters of the five samples was evaluated. HPTLC analysis of the samples against the standard solutions of Gallic acid (GA) and Tannic acid (TA) was carried out with Toluene: Ethyl acetate: Formic acid (2:5:1.5) as the mobile phase. Antioxidant activity was evaluated using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay against the standard Ascorbic Acid (AA). The HPTLC analysis of all five samples showed similar patterns with respect to their peaks and intensities while showing peaks corresponding to the peaks of standards. A high level of anti-oxidant activity was found in the samples varying in the decreasing order of S_3 (1:2:4) > S_1 (1:1:1) > S_4 (1:2:2)> S_2 (1:2:3) > S_5 (1:2:4 Fruits). In spite of the high level of antioxidant activity observed with all five samples, the most pronounced level of antioxidant activity was found in S_3 powder sample. Further studies need to be carried out on bioactivity studies to evaluate the therapeutic efficacy of different compositions of *Triphala* powder.

Keywords: *Triphala* powder, Ayurveda authentic texts, HPTLC, Anti-oxidant activity, DPPH assay

Introduction

Triphala constitutes a polyherbal formulation deeply rooted in both Ayurveda and Sri Lankan Traditional Medical practices. *Triphala,* a term derived from the Sanskrit language where "*tri*" signifies "three," and "*Phala*" translates to "fruits" in English, conveys a specific reference to a compound comprising the dried pericarps of three botanical constituents: *Haritaki (Terminalia chebula), Vibhitaki (Terminalia bellirica)* both of which belong to the Combretaceae family and *Amalaki (Phyllanthus emblica)* belong to the Euphorbiaceae family.

This herbal formulation, combining the therapeutic properties of these three fruits, has holistic and numerous health benefits. *Triphala* possesses a myriad of health-enhancing qualities attributable to its various properties, including but not limited to its anti-diabetic, antioxidant, antibacterial, anti-inflammatory, free radical scavenging, immune modulating, appetite stimulation, gastric hyperacidity reduction, prevention of dental caries, antipyretic, analgesic, antibacterial, antimutagenic, wound healing, anticarcinogenic, antistress, adaptogenic, hypo- glycemic, anticancer, hepatoprotective, chemoprotective, radio-

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protective, and chemo preventive effects[1]. This polyherbal formulation is proven to enhance proper digestion and absorption of food, improves circulation, increase production of red blood cells and hemoglobin, lowers serum cholesterol levels, relax bile ducts, prevent immune senescence, maintain homeostasis of the endocrine system1. Previous studies have proven the potential of *Triphala* in weight reduction and reduction of body fat. Furthermore, *Triphala* also decreased total cholesterol, triglycerides, and low-density lipoprotein cholesterol[2]. A clinical study carried out on noninsulin-dependent diabetes mellitus patients have clearly shown that supplementation with 5.0 g of *Triphala* powder for 45 days significantly lowered blood glucose levels[3]. Reduction of abdominal pain, hyperacidity, constipation, mucous, and flatulence while improving the frequency, yield, and consistency of stool was observed in a clinical trial that investigated the use of *Triphala* in patients with gastrointestinal disorders[4]. Furthermore, *Triphala churna* has shown to have multiple beneficial effects in diabetic neuropathy which may be attributed to reduced oxidative stress, inhibition of inflammatory cytokines and increased expression of Nerve Growth Factor in rats[5]. Several previous studies proven that *Triphala* exerts an antineoplastic effect on many cancer cell lines, including cancers of the prostate, colon, breast, and pancreas[6-8]. Based on animal studies carried out on stress, researchers have determined that *Triphala* has a potential of protecting against cold-induced stress and reversed stress-induced behavioral alterations and biochemical changes such as increased lipid peroxidation and corticosterone levels[9]. In addition to cold induced stress, previous studies prove that *Triphala* also prevented noise-induced stress[10]. In vitro studies the *Triphala* extract exhibited significant free radical scavenging activity on hydrogen peroxide- induced cell damage and senescence proving that T*riphala* extract exerted highly protective antiaging effects on human skin cells[11].

Antioxidants are crucial for maintaining overall health and preventing oxidative damage. The popularity of the antioxidant activity exhibited by *Triphala* is well-established through various research findings. Antioxidant effects of *Triphala* is considerably more significant to help maintain eye health. *Triphala* serves as a reservoir of vitamin C and a variety of flavonoids. Significant restoration of glutathione levels in eye lenses was observed in a study where *Triphala* was used as a pretreatment in selenite-induced cataracts in mice. Furthermore, *Triphala* increased the activities of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione-S-transferase, and glutathione peroxidase, in the eye lenses[12].

Previous research studies have discovered that tannins, gallic acid, ellagic acid, and chebulinic acid as the major chemical constituents of *Triphala* which are potent antioxidants responsible for the observed immunomodulatory activity of Triphala[13-15]. Along with that, studies have also proven that *Triphala* contains many other bioactive compounds like flavonoids (e.g., quercetin and luteolin), saponins, anthra-quinones, aminoacids, fattyacids, and various carbohydrates[16]. Furthermore, depicting the Anti-oxidant properties, this formulation is proven to contain *Triphala*-derived polyphenols such as chebulinic acid which is transformed by the human gut microbiota into bioactive metabolites, exhibiting its promising in vitro potential for the prevention of oxidative damage[17].

Numerous compositions of this blend have been extensively mentioned within Authentic Ayurveda texts, including Charaka Samhita, Susruta Samhita, Bhavaprakasha, Yogaratnakara, Madanapala Nighantu, Kaiyadeva Nighantu, Chakradatta, Sharangadhara Samhita, and through oral transmission within the Ayurveda tradition. Susruta Samhitha mentions that *Triphala* pacifies *Kapha* and *Pitta dosha*, effective in curing diabetes, skin diseases, promotes vision, enhances digestive fire and cures intermittent fevers[18]. According to Yogarathnakara the combination, *Triphala* is beneficial in edema/ inflammation, diabetes, intermittent fevers, improve appetite, alleviates *Kapha* and *Pitta* and *Kushta* (skin diseases) and has *Rasayana* (rejuvenation) and overcomes eye

diseases[19]. Bhavaprakasha mentions that reducing *Kapha* and *Pitta*, useful in curing urinary diseases and glycosuria, potential laxative, beneficial for eyes, improving appetite, promoting taste sensation and curing Malarial fevers as the properties of Triphala[20]. In Charaka Samhitha, *Triphala* is mentioned under *Rasayana chikitsa* (Rejuvenation Therapy)[21]. According to Acharya Charaka a person undergoing rejuvenation therapy attains long life, good memory, intellect, helps to be free from diseases, maintains youth, promotes excellence of luster, complexion and voice, promote excellent potentiality of the body, senses respect and brilliance [22].

The present study endeavors to compare the chemical characteristics of distinct compositions of *Triphala* powder as mentioned in various Ayurveda authentic texts. Specifically, this study conducted a comparative analysis of the High-Performance Thin-Layer Chromatography (HPTLC) profiles and evaluated and compared the antioxidant activity of distinct compositions of *Triphala* powder, as specified in diverse Ayurveda authentic texts.

Materials and methods

Identification of different compositions of Triphala powder

The identification of various compositions of *Triphala* powder was achieved following a comprehensive literature review in the Authentic Ayurveda texts: Charaka Samhita, Susruta Samhita, Bhavaprakasha, Yogaratnakara, Madanapala Nighantu, Kaiyadeva Nighantu, Chakradatta, Sharangadhara Samhita, and through oral transmission within the Ayurveda tradition.

Preparation of Triphala powder of different compositions

The fruits of *Terminalia chebula* (TC), *Terminalia bellirica* (TB), and *Phyllanthes emblica* (PE) were purchased from the local market and authenticated from the Department of Ayurveda Pharmacology, Pharmaceutics and Community Medicine, Faculty of Indigenous Medicine, University of Colombo, Rajagiriya. The ingredients were cleaned, washed and dried. The resultant dried materials were finely pulverized (sieve size No. 120), yielding five samples, S_1 (1:1:1), S_2 (1:2:3), S_3 (1:2:4), S_4 (1:2:2) for the powders of TC: TB: PE respectively and S_5 – 1:2:4 for the fruits of TC: TB: PE, in accordance with the specified compositions documented in authentic Ayurveda texts (Table 1).

Preparation of ethanol extracts of Triphala powder of different compositions

Ethanol extracts were prepared from 5.0 g of each sample with 150 mL of ethanol using Soxhlet apparatus. The resultant extract was concentrated and dried at 40^0C using Rotary evaporator to get the Ethanol extracts of *Triphala* powder of different compositions.

Preparation of standards: Gallic acid (GA) and Tannic acid (TA) for HPTLC

Gallic acid (GA) and Tannic acid (TA) were purchased from the local market. A precisely measured quantity of 5 mg of each GA and TA was individually dissolved in 2.5 milliliters of methanol, thereby yielding concentrations of 2 mg/mL for each respective substance.

The High-Performance Thin-Layer Chromatography (HPTLC) analysis

In HPTLC, the Stationary phase was Aluminum plates precoated with Silica gel 60 F 254, size: 10cm x10cm (Merck, Germany). The mobile phase composition was Toluene: Ethyl acetate: Formic acid (2:5:1.5)[27]. Spotted the 5 samples against the standard solutions of Gallic acid (GA) and Tannic acid (TA) at concentrations of 2 mg/mL and allowed to dry at room temperature. After saturation of the twin trough chamber for 30 minutes at room temperature the plate was developed in the solvent system and allowed to dry at room temperature. The comparability of Retention factor (Rf) values among all samples and standards was observed under UV illumination at wavelengths of 254nm and 366nm using win CATS software.

Table 1: Different compositions of *Triphala* powder in various Ayurveda authentic texts

Sample	Form	Composition of three ingredients			Original Source
		TC	TB	PE	
S_1	Powder	1	1	1	1) Susruta Samhitha, *Sutrasthana*[18] 2) Bhavaprakahsa, *Purva Khanda*[20]
S_2	Powder	1	2	3	Oral Tradition
S_3	Powder	1	2	4	1) Yogarathnakara, Basic concepts of Ayurveda[19] 2) Madanaphala Nighantu, *Abhayadi varga*[23] 3) Kaiyadeva Nighantu, *Oshagivarga*[24] 4) Chakradatta, *Rasayanadhikara*[25]
S_4	Powder	1	2	2	Oral Tradition
S_5	Fruits	1	2	4	1) Charaka Samhitha, *Chikithsastana*[21] 2) Sharangadhara Samhitha, *Madhyama Khanda*[26]

Determination of antioxidant activity of the different compositions of Triphala

Preparation of concentration series for samples and the standard: Ascorbic Acid (AA) to analyze the antioxidant activity

A concentration series for the five distinct samples and the standard Ascorbic Acid (AA) was precisely made over a range spanning from 5 parts per million (ppm) down to 0.6 ppm. This was obtained through the dissolution of both the five samples and the standard Ascorbic Acid (AA) in methanol.

To ensure reliability, this concentration series was triplicated, reinforcing the consistency and accuracy of the analytical measurements and assessments.

Analysis of the Antioxidant Activity- 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

Antioxidant capacity of all samples S_1, S_2, S_3, S_4, S_5 and the standard AA were determined through the evaluation of free radical scavenging effect on the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical[28]. A freshly prepared DPPH solution was made up to 0.5mg/mL concentration with methanol and the solution was mixed with sample series /AA solution series in methanol in 96 well plate. The entire experimental setup was then incubated in dark conditions at room temperature for a duration of 10 minutes. Upon the completion of the incubation period, the absorbance of the reaction mixtures was measured at a wavelength of 517 nanometers (nm). This quantification was performed using a UV visible spectrophotometer (Multiskan SkyHigh UV/Vis), equipped with specialized software Skanlt TM. Radical scavenging activity which was expressed as the inhibition percentage was calculated using the following formula[29];

$$\text{Percentage Inhibition} = \frac{Ac - As}{Ac} \times 100\%$$

Ac -Absorbance of the control, As - Absorbance of the sample.

Results

HPTLC analysis

The HPTLC profiles revealed the presence of nine distinct peaks exhibiting similar patterns in terms of peak profiles and their respective intensities in all samples, characterized by a range of Retention factor (Rf) values spanning from 0.01 to 0.88. The Rf values corresponding to the standards, GA and TA were identified as 0.88 and 0.73 respectively. In each of these samples, similar peaks were observed, aligning closely with the characteristic peaks demonstrated by the standards of GA and TA.

Figure 1 to 5 shows the developed HPTLC and TLC Profiles with peak densitogram of ethanol extracts of S_1, S_2, S_3, S_4, S_5 respectively. Figure 6 and 7 shows the TLC chromatogram under 366 and 254nm respectively.

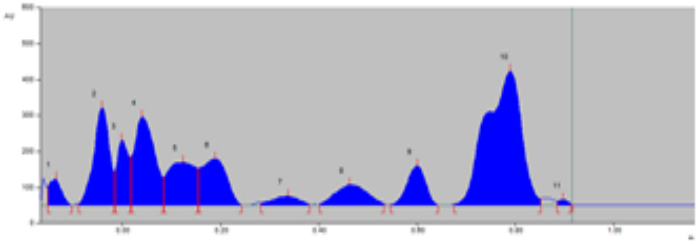

Fig. 1: HPTLC and TLC Profile with peak densitogram of ethanol extracts of S_1

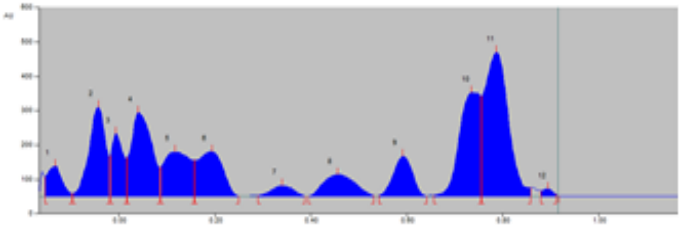

Fig. 2: HPTLC and TLC Profile with peak densitogram of ethanol extracts of S_2

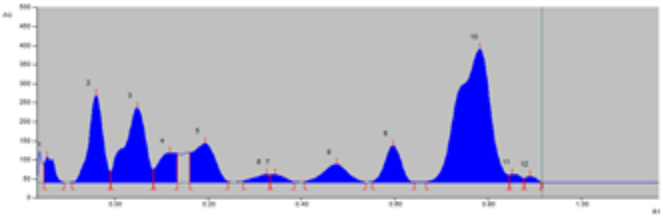

Fig. 3: HPTLC and TLC Profile with peak densitogram of ethanol extracts of S_3

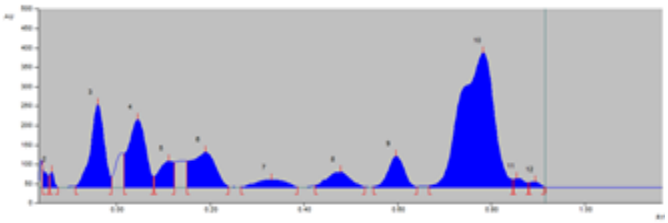

Fig. 4: HPTLC and TLC Profile with peak densitogram of ethanol extracts of S_4

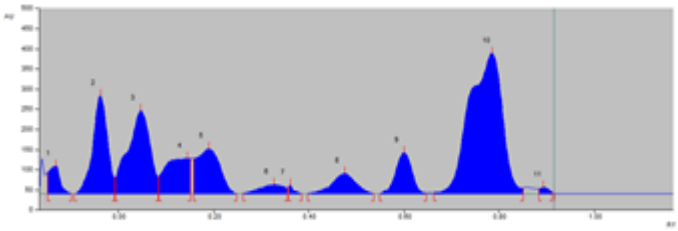

Fig. 5: HPTLC and TLC Profile with peak densitogram of ethanol extracts of S_5

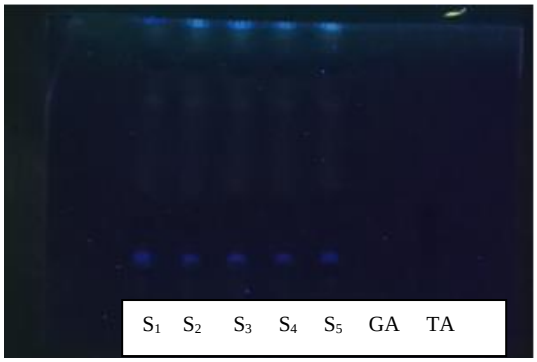

Fig. 6: TLC chromatogram under 366nm

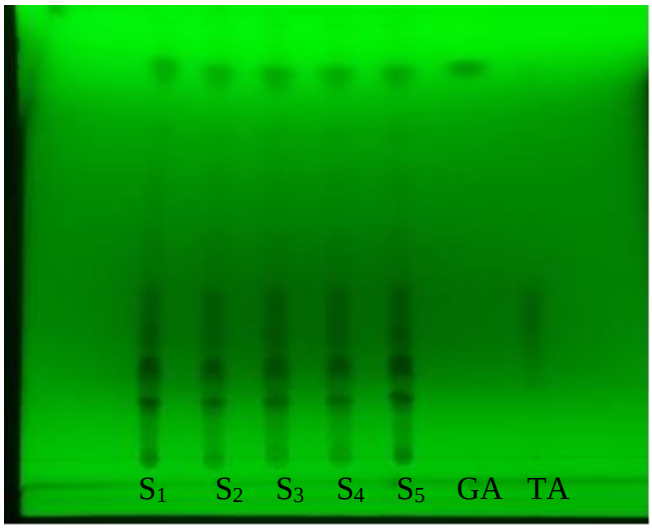

Fig. 7: TLC chromatogram under 254nm

Table 2 shows the Rf value (Retardation Factor), AU value (Area Under curve) of the 5 samples. The highest area distribution is shown a particular substance referred to as the 10th peak of S_1 (1:1:1), S_3 (1:2:4), S_4 (1:2:2) powders respectively and S_5 (1:2:4 for the fruits) and the 11th peak of S_2 (1:2:3) for the powders of *Triphala.*

Antioxidant activity of the different compositions of Triphala

DPPH Assay

Table 3 shows the ability of the 5 samples to scavenge the DPPH free radical from the obtained respective percentage inhibition values at different concentrations.

Table 4 shows the shows ability of Ascorbic acid to scavenge the DPPH free radical at different concentrations.

Table 5 shows the IC_{50} values of the *Triphala* samples and Ascorbic acid. Among the 5 samples, the lowest IC_{50} (1.32 ± 0.29 ppm) showed from S_3 sample and it varied in the ascending order ($S_3 < S_1 < S_4 < S_2 < S_5$). IC_{50} value of Sample S_3 is very much close to the standard IC50 value.

Table 2: Peak distribution and area distribution of samples in HPTLC

Peak	S_1 (1:1:1)	S_2 (1:2:3)	S_3 (1:2:4)	S_4 (1:2:2)	S_5 (1:2:4 Fruits)
4	0.02-0.08 Rf 6964.1AU	0.02-0.09 Rf 7264.4 AU	0.08 - 0.13 Rf 2036.5 AU	0.01 - 0.08Rf 4439.0 AU	0.09 - 0.15 Rf 3173.2 AU
5	0.09-0.15 Rf 4573.0AU	0.09-0.16 Rf 4967.0 AU	0.16 - 0.24 Rf 3375.1 AU	0.08 - 0.12Rf 1474.6 AU	0.16 - 0.25 Rf 3762.2 AU
6	0.16-0.24 Rf 4571.6AU	0.16-0.25 Rf 4692.0 AU	0.28 - 0.33 Rf 480.0 AU	0.15 - 0.24Rf 3232.5 AU	0.26 - 0.35 Rf 803.3 AU
7	0.28-0.38 Rf 926.4AU	0.29-0.39 Rf 971.4 AU	0.33 - 0.38 Rf 426.1 AU	0.26 - 0.39Rf 898.5 AU	0.36 - 0.38 Rf 158.1 AU
8	0.40-0.53 Rf 2556.0AU	0.40-0.53 Rf 2881.9 AU	0.41 - 0.54 Rf 1749.3 AU	0.42 - 0.53Rf 1370.2 AU	0.40 - 0.54 Rf 1745.4 AU
9	0.55-0.64 Rf 2589.7AU	0.54-0.64 Rf 3210.8 AU	0.55 - 0.64 Rf 2290.9 AU	0.55 - 0.64Rf 1910.0 AU	0.55 - 0.65 Rf 2416.6 AU
10	0.68-0.85 Rf 18716.9AU	0.66-0.75 Rf 9051.5 AU	0.67 - 0.85 Rf 16620.0 AU	0.67 - 0.85Rf 16960.8 AU	0.66 - 0.85 Rf 16736.0 AU
11	0.88-0.95 Rf 207.7AU	0.76-0.86 Rf 14141.9 AU	0.85 - 0.88 Rf 345.5 AU	0.85 - 0.88Rf 373.0 AU	0.88 - 0.91 Rf 212.8 AU
12		0.88 - 0.91 Rf 333.0 AU	0.88 - 0.92 Rf 247.4 AU	0.88 - 0.91Rf 214.6 AU	

Table 3: Percentage of Inhibition of different samples (S_1, S_2, S_3, S_4, S_5)

Concentration (ppm)	S_1 Inhibition %	S_2 Inhibition %	S_3 Inhibition %	S_4 Inhibition %	S_5 Inhibition %
5	61.89	63.93	74.35	63.53	57.29
4	59.95	54.11	70.49	62.73	53.36
3	54.51	57.04	63.11	55.44	49.24
2	52.07	47.83	58.84	52.27	42.28
1	47.74	46.33	50.32	48.77	37.02
0.8	46.37	41.54	45.73	44.13	36.5
0.6	45.50	42.12	39.45	35.77	38.25

Table 4: Percentage Inhibition of Ascorbic acid

Concentration(ppm)	Percentage Inhibition	Average
4	72.10784713	72.11
3	62.83760059	62.84
2.5	57.55104515	57.55
2	55.32973414	55.33
1.5	51.57644045	51.58
1	47.01771475	47.02
0.5	44.72366102	44.72
0.1	45.84138189	45.84

Table 5: IC_{50} Values of samples *Triphala* and standard

Sample /Standard	IC $_{50}$ value
Ascorbic Acid	1.17 ± 0.05 ppm
S1 (1:1:1 powder)	1.67 ± 0.16 ppm
S2 (1:2:3 powder)	2.25 ± 0.47 ppm
S3 (1:2:4 powder)	1.32 ± 0.29 ppm
S4 (1:2:2 powder)	2.01 ± 0.64 ppm
S5 (1:2:4 Fruits)	3.40 ± 0.22ppm

Discussion

HPTLC analysis showing presence of nine distinct peaks exhibiting similar patterns and respective intensities in S_1, S_2, S_3, S_4, and S_5 reveal that all the samples consist of similar chemical substances.

Antioxidant activity refers to the ability of certain substances, known as antioxidants, to neutralize or counteract the damaging effects of molecules called free radicals within the body. Based on the values obtained from Percentage Inhibition to scavenge the DPPH free radical and Half Maximal Inhibitory Concentration (IC_{50}) which determines the concentration at which a substance exerts half of its maximal inhibitory effect, it is clear that all five samples: S_1, S_2, S_3, S_4, and S_5, exhibited pronounced and high levels of antioxidant activity in DPPH Assay. Specifically, it is observed that the levels of antioxidant activity exhibited a descending order as follows: S_3 (1:2:4 powder of TC: TB:PE) > S_1 (1:1:1 powder of TC: TB:PE) > S_4 (1:2:2 powder of TC: TB:PE) > S_2 (1:2:3 powder of TC: TB:PE) > S_5 (1:2:4 Fruits of TC: TB:PE). The highest antioxidant potential was identified in sample 3 (IC_{50}=1.32 ± 0.29 ppm) with a level that closely approaches the antioxidant activity observed in the standard ascorbic acid (IC_{50}=1.17 ± 0.05 ppm). This heightened antioxidant potency compared to other samples can be attributed to the fact that S_3 (1:2:4 powder of TC: TB: PE) possesses the highest concentration of *Amalaki*, one of constituent fruits in *Triphala*. Charaka Samhitha, Sutrasthana mentions *Amalaki-Phyllanthus emblica* as one ingredient among the *Vayasthapana dashaka* (10 longevity promoters).[30] S_1 (1:1:1) has the potential of Kapha and Pitta pacifying properties and S_3 (1:2:4) is rich with *Rasayana* (Rejuvenation) action which destroys senility, diseases and promote longevity due to the highest concentration of *Amalaki*.

It is recommended that future research could be done on bioactivity studies to assess the therapeutic efficacy of different compositions of *Triphala* powder based on Ayurveda Authentic Texts.

Conclusion

From the present study it can be concluded that, even though the chemical characteristics of various formulations of *Triphala* powder, as specified in diverse Ayurveda authentic texts exhibited notable similarity, the highest antioxidant activity was found in Sample 3 (1:2:4 powder of TC: TB:PE).

Conflict of Interest

The authors have declared that there is no conflict of interest.

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Comparative evaluation of *Triphala* mouthwash with three different proportion combination

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Abstract

Oral health is an essential component of overall health and a satisfactory quality of life. The oral disease is termed as *Mukharoga* in Ayurveda. *Gandusha* is mentioned as one of the major therapies in *Ayurveda* medicine for both preventing and treating oral diseases. *Acharya Sharangadara* mentioned *Triphala* as a *Gandusha* and it consists of three myrobalans, which are *Terminalia chebula*, *Terminalia bellerica*, *Phyllanthus emblica. Triphala* is used as equal and unequal proportions. In the present study, *Triphala* mouth wash (TM) is prepared in the form of *Kwatha* (Decoctions) according to the Ayurveda Bhaisajyaratnavali. Then the same ingredients were used with different propotions to prepare the decoctions. They were considered as mouth wash based on the comments of Sharangadhara. The prepared decoctions were comparatively analyzed with standardization parameters. Decoctions were prepared in 3 ratios as follows: 1:1:1(TM1), 1:2:3 (TM2) and 1:2:4 (TM3) and assessed the organoleptic, physiochemical and phytochemical. Moreover, pharmacological properties were also evaluated using the authentic text books and the modern research findings. Dichlormethane fractions of *Thriphala* decoction in all three proportions were used to develop TLC and HPTLC fingerprints equivalent to gallic acid and tannic acid under the Toluene: Ethyl acetate: formic acid 2:5:1.5 solvent system. pH values of TM1, TM2 and TM3 were 3.98, 3.92, 3.96 respectively. The Foaming index was found to be less than 100 in all the three proportions. Phytochemical screening shows the presence of tannins, saponins, flavonoids etc. The TLC and HPTLC fingerprints showed the presence of tannic and gallic acid as active ingredients when visualized under UV at 254 and at 366 nm and the *Triphala* mouth wash prepared in 1:2:3 ratio showed the highest Rf values. Based on the pharmacological properties and actions, all the three proportions of *Thriphala* mouth wash can be used for oral diseases, but the effectiveness of therapeutic action may vary slightly according to the proportions of decoction.

Keywords: Oral diseases, *Gandusha*, Decoction, *Triphala* mouthwash, Different proportions

Introduction

Ayurveda is a science of life which engages with prevention and treatment of various diseases and promoting health in healthy individuals. It is a healing gift to us from the ancient enlightened Vedic culture. Oral health is an important and integrated component of overall health. It is also recognized as an essential part of the quality of life[1,2].

Oral illnesses continue to be a serious health issue and a highly prevalent group of pathologies in the world. Oral health refers to the state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal disease, tooth decay, tooth loss, and any other disorders that limit the individual's oral function. According to the World Health Organization (WHO) Global Oral Health Status Report (2022), oral illnesses are the most prevalent non-communicable diseases globally, affecting almost half of the world's population (45% or 3.5 billion people worldwide). Poor oral health can affect the individual's capacity in biting, chewing, smiling, speaking and psychosocial

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wellbeing. Oral diseases disproportionally affect people in developing countries. Some of the oral diseases are cavities, periodontal (gum) diseases, mouth sores, interdental bleeding, bad breath and gingivitis etc[3].

The illness of the oral cavity is termed as *Mukharoga* in Ayurveda. *Mukha* is considered as one of the *Nava dwara* and it is the most important part of the body. *Acharya Charaka* has mentioned sixty four *Mukha Roga* which occur at seven locations, such as lips, gums, teeth, tongue, palate, throat and oral cavity. According to *Madhava Nidana*, excessive intake of meat of marshy creatures, milk, curd, fish etc. causes an abnormal increase in the three *Doshas* with the predominance of *Kapha* and productive diseases of the mouth[4,5,6].

There are several treatment procedures mentioned in *Ayurveda* classics to avoid and eradicate *Mukharogas*, and *Gandusha* is one among of them explained by *Acharya Charaka* under the *Dinacharya* (daily routine), *Acharya Sustruta* under the *Anagatabadha pratishedha* and *Acharya Vagbhata* under the *Gandushadi Vidhi*. *Gandusha* is the process of holding any decoction, oil, or any medicated liquid in the mouth to its full capacity without moving it. Regular practice of *Gandusha* will help to increase good oral hygiene by keeping the cleansing action and increasing the defense mechanism in the oral cavity. *Acharya Charaka* in Charaka Samhita mentioned the benefits of *Gandusha darana* such as strength of the jaws, voice and roots of the teeth, relief from the dry throat, lip cracking, dental caries, pain and discomfort, increased capacity for chewing. Increase the taste and appetite of food.

Mouthwashes are solutions or liquids used to rinse the mouth and they have several benefits, such as to destroy bacteria, to act as an astringent, to deodorize, to prevent dental caries and to reduce or eliminate plaque accumulation.[7, 8, 9, 10]

In Ayurveda, *Triphala* (*tri* –three, *phala* –fruit) is a well-known polyherbal formulation and it has been used in *Ayurveda* for over 2000 years. It is used extensively in Ayurveda and consists of three myrobalans, such as *Haritaki (Aralu), Viḅhitaki (Bulu), Amalaki (Nelli)*. It provides therapeutic value for several diseases and oral pathologies are one of those. It mainly alleviates *Kapha* and *Pitta* and it is among the most ancient and common of the *Ayurvedic* remedies. *Triphala* contains various powerful antioxidants and bio-active substances which are good for oral health. Also, *Triphala* is claimed to have antibacterial and antiviral effects. Therefore, it is commonly used as a mouthwash (*Gaṇḍuṣha*) in *Ayurveda*.

Aralu– Terminalia chebula

Haritaki is also known as *Harar*, *Harra*, *Hirda* and Myrobalan and it belongs to the Combretaceae family. *Haritaki* was named as king of medicine. It is mainly used for constipation, respiratory problems, certain skin diseases, eye diseases etc. and *Bhavamishra* mentioned seven different varieties of *Terminalia Chebula* .It has several pharmacological activities, such as Anti-diabetic, Anti-carcinogenic,Anti-viral, Anti-fungal, Anti-oxidant, Anti-inflammatory, Anti-bacterial activity.

Bulu – Terminalia bellerica

Terminallia bellerica is called *Vibhitaki* in Sanskrit, which means fearless and it belongs to the Combretaceae family. *Acharya Charaka* mentioned *Vibhitaki* in the *Jvarahara* and *Kasahara* group of drugs and he also indicated it for *Rasa*, *Rakta*, *Mamsa* and *Meda Vikaras*. Its pharmacological activities are Analgesic activity, Anti-diarrhea, Anti – microbial, Anti-oxidant, Anti-cancer, Wound healing and Anti – ulcer activity etc.

Nelli – Phyllanthus emblica

Phyllanthus emblica or Indian gooseberry is commonly known as *Amla* (Sanskrit *Amalaki*) and it is a medium sized deciduous tree of the family Euphorbiaceae. *Phyllanthus emblica* is one of the richest sources of natural vitamin c, amino acids and minerals. It consists of Anti-oxidant, Anti-microbial, Anti-cancerous, Anti-diabetic, Anti-ageing, Anti-ulcer, Anti-inflammatory, Antifungal, Anti-viral activities. [11,12,13,14]

In Ayurveda Bhaiṣajyaratnavali, the preparation method of *Tripala kvata* was mentioned but the proportions of each ingredient are not given. Therefore, in this study, *Thriphala* Decoction as a mouth wash was prepared from different proportions, such as 1:1:1 ratio, 1: 2:3 ratio, and 1:2:4 ratio, and comparatively analysis of different proportions of *Triphala* mouth wash for oral diseases.[15]

The present study was aimed on comparatively evaluation of *Triphala* mouthwash with three different proportion combination, and specific objectives is to compare the TLC and HPTLC fingerprinting profiles of *Triphala* mouth wash, which is prepared in 3 ratios.

Materials and Methods

Collection and authentication of plant materials

Herb authentication is a quality assurance that make sure the correct plant species and plant parts are used as raw materials for herbal medicine. The correct process of authentication for herbal raw materials is more important to the safety and efficacy of herbal medicines.

Fruits of *Terminalia chebula* (Combretaceae), *Terminalia belerica* (Combretaceae) and *Phylanthus emblica* (Euphorbiaceous) were purchased from a local registered *Ayurveda* shop in Colombo city, Western province, Sri Lanka and authenticated by the Department of *Dravyaguna vignana* and *Swasthavritta* Faculty of Indigenous Medicine, University of Colombo. The contaminants of the raw materials were removed manually, washed with water, and shade dried (Figure 1, 2 and 3).

Fig. 1*:* Collected and Authenticated *Haritaki*

Fig. 2: Collected and Authenticated *Vibhitaki*

Fig. 3: Collected and Authenticated *Amalaki*

Preparation method of Triphala mouth wash

Seeds of individual fruits were removed and the dried fruit pulp was separately crushed in to small particles using *Ullukala yantra.* Then *Triphala* decoction was prepared according to the traditional decoction preparation method by mixing *Haritaki, Vibhitaki, Amalaki* in ratio of 1:1:1 (TM1), 1:2:3 (TM2), 1:2:4 (TM3) in separate containers and boiled with 1920ml of water under mild flame to reduce the volume up to 240ml.

Analytical study[16, 17, 18, 19]

Organoleptic properties

Organoleptic evaluations were tested according to the color, odor, and taste parameters by visual examination (Figure 4).

Physiochemical parameters

The pH values were measured using a bench digital pH meter. A total of 50ml of each sample were inserted in to separate beakers and they proceeded with the analysis. Each sample of *Triphala* mouth wash was measured three times. The final pH was set as the arithmetic mean of the values recorded.

Table 1: Ingredients of *Thripala* decoction

S. No	Ingredients	Botanical name	Part used	Quantity taken		
				1:1:1	1:2:3	1:2:4
1	*Haritaki*	*Terminalia chebula*	Fruit	20g	10g	8.6g
2	*Vibhitaki*	*Terminalia belerica*	Fruit	20g	20g	17.14g
3	*Amalaki*	*Phylanthus emblica*	Fruit	20g	30g	25.8g
4	Water			1920ml	1920ml	1920ml

Fig. 4: Samples of *Triphala* mouthwash in different proportions

Determination of Foaming Index

Pour the decoction in to 10 stoppered test tube as 1 ml, 2ml, 3ml, etc. up to 10ml and adjust the volume up to 10 ml by adding sufficient quantity of distilled water Shake the test tube for 15 sec in length wise motion (Two shake per second) and keep the test tube to stand for 15 min and measure the height of the foam. This procedure was done for each proportion of dichloromethane fraction

- If the measure of foam height in every tube appears to be less than 1cm, then the foaming index is less than 100.
- If the measure of foam height in every tube appears to be more than 1cm, then the foaming index is over 1000.

Preparation of extraction

Freshly prepared *Triphala* mouthwash of different proportioned forms was added separately (100ml from each) to a separating funnel containing 50 ml of dichloromethane, mixed well and kept for 20 min. After that, the Dichloromethane layer was separated of each proportion separately and put in the hot air oven to evaporate the moisture.

Phytochemical parameters

Dried dichloromethane fractions of each proportioned forms (TM1, TM2, TM3) were again dissolved in dichloromethane separately. The prepared test extracts were analyzed qualitatively for the presence of various phytoconstituents such as tannin, sapponins, phenols, Carbohydrates, Flavonoids, alkaloids

Determination of the presence /absence of tannins

Extract was added with a few drops of $Fecl_3$ (10%) solution. Tannins indicate a solution that is greenish grey or dark blue in color. This procedure was done for each proportion of extract.

Determination of the presence /absence of sapponins

Tripala decoction was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Stable persistent froth was taken as an indication for the presence of saponins. This procedure was done for each proportion of *Triphala* decoction.

Determination of the presence /absence of Phenols (Vanillin test)

2ml of Extract were added with a few drops 10% vanillin in ethyl alcohol and conc. HCL Appearance of red color indicate the presence of phenols. This procedure was done for each proportion of extract.

Determination of the presence /absence of Carbohydrates (Molisch's test)

2ml of Extract were added with 2 ml of Molisch's test reagent and shaken well. To this another 2ml of concentrated sulphuric acid was added carefully through the sides of the test tube. Appearance of a reddish violet ring at the interphase indicate the presence of carbohydrates. This procedure was done for each proportion of extract.

Determination of the presence/absence of Flavonoids

5ml of dilute ammonia solution was added to 5ml of extract followed by the addition of conc.H_2SO_4. Appearance of yellow color indicates the presence of flavonoids. This procedure was done for each proportion of extract.

Determination of the presence /absence of alkaloids (Mayer's test)

2ml of Extract were added with 2ml of Mayer's reagent (potassium mercuric chloride) and mixed well. Appearance of a yellow colored precipitate indicates the presence of alkaloids. This procedure was done for each proportion of extract.

Development of TLC and HPTLC fingerprints

Preparation of standard solution of Gallic acid

Gallic acid stock solution was prepared by dissolving 7.5mg of accurately measured Gallic acid in methanol and adjust the volume up to 100ml with methanol.

Develop spotted TLC plate

Dried dichloromethane fractions of different proportioned forms (TM1, TM2, and TM3) were again dissolved in 5ml of dichloromethane separately and spotted on a TLC plate along with Gallic acid and Tannic acid as standards. The TLC fingerprint profile was developed for all fractions using toluene, ethyl acetate and formic acid at a ratio of 2:5:1.5 v/v. The plate was visualized under UV radiation and HPTLC fingerprint patterns were observed by using HPTLC scanner (Figure 5).

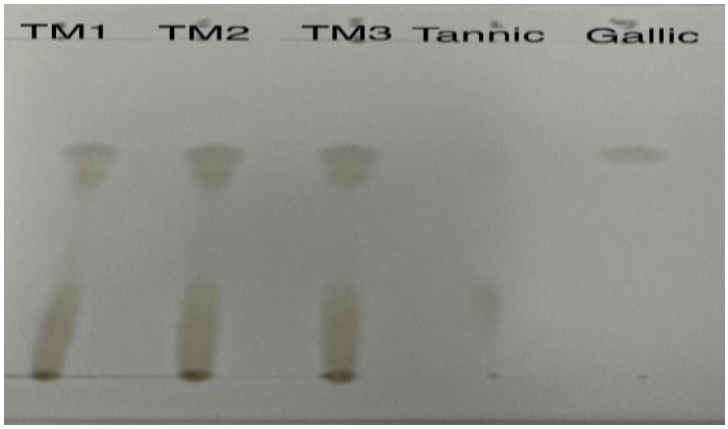

Fig. 5: Spotted TLC plate

Results

Organoleptic characters

Organoleptic character for the raw materials was done with samples taken from the Ayurveda shop. The results obtained and the standard values are given Table 2.

According to the organoleptic properties**,** *Tikta Kashaya* taste is the most prominent taste in all three proportions, and compared to the others, TM2 has the strongest characters compared with other two samples.

Physiochemical Screening

Determination of pH

The pH of *Triphala* mouthwash in different proportions were determined separately and the pH value obtained is tabulated in Table 3.

The pH value of all three samples shows an acidic nature, and TM1 showed slightly higher value and compared with the sample TM 2 showed the lowest value.

Determination of Foaming Index

The foaming index was calculated and expressed according to the WHO recommendations and of all 3 samples was found to be less than 100.

Phytochemical parameters

Preliminary phytochemical analysis for all three proportions revealed the positive results for total 6 compounds such as tannin, saponin, phenol, carbohydrates, flavonoid, and alkaloid (Table 5).

Table 2: Organoleptic characters

S. No.	Sample	Parameters		
		Colour	Taste	Odour
1.	1:1:1 ratio (TM1)	Brown	*Tikta, Kashaya*+	Characteristic
2.	1:2:3 ratio (TM2)	Blackish Brown	*Tikta, Kashaya*+++	Characteristic
3.	1:2:4 ratio (TM3)	Dark Brown	*Tikta, Kashaya*++	Characteristic

Table 3: Determination of pH

S: No	Sample	pH
1	1:1:1 ratio (TM1)	3.98
2	1:2:3 ratio (TM2)	3.92
3	1:2:4 ratio (TM3)	3.96

Table 4: Determination of Foaming Index

	1:1:1 ratio	1:2:3 ratio	1:2:4 ratio
Foaming Index	Less than 100	Less than 100	Less than 100

Table 5: Determination of phytochemical parameter

S. No.	Phytochemical constituents	Name of the test	Observation	Result		
				1:1:1 ratio	1:2:3 ratio	1:2:4 ratio
1	Tannin	$FeCl_3$ test	Blue colour appear	+	+	+
2	Saponin	Foam test	Persistent froth appears	+	+	+
3	Phenol	Vanillin test	Red color appears	+	+	+
4	Carbohydrate	Molisch's test	Reddish violet ring appears	+	+	+
5	Flavonoid	Ammonia test	Yellow color appears	+	+	+
6	Alkaloid	Mayer's test	Yellow color appears	+	+	+

TLC and HPTLC Fingerprinting

TLC analysis of *Triphala* mouthwash in different proportions revealed the presence of various bioactive compounds and among them, according to the literature Tannin and Gallic Acid are the major bioactive present. Therefore, in the present study the TLC was run with equivalent to the tannin and gallic acid and it showed the parallel bands in all three samples. Under the 366 nm wave length also showed the presence of the tannin and gallic acids (Figure 6 and 7).

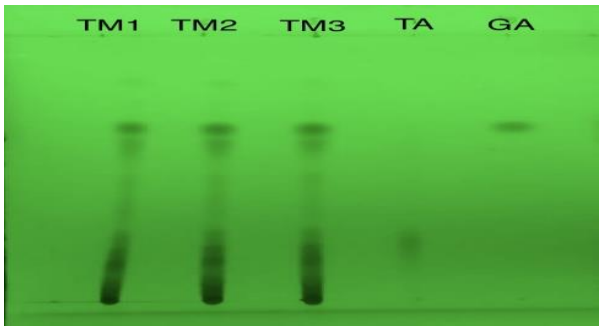

Fig. 6: TLC photo documentation under 256nm UV light

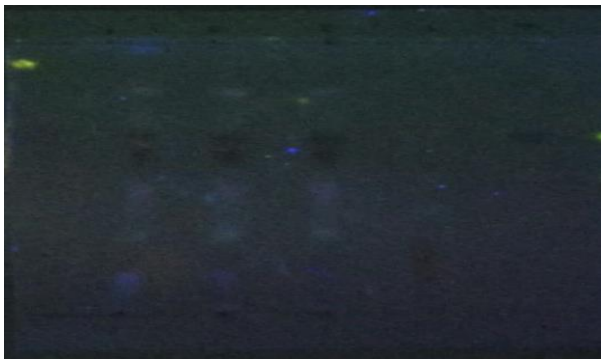

Fig. 7: TLC photo documentation under 366 UV light

In the ratio of the 1:1:1 showed the 5 peaks with the Rf values of -0.07, 0.01, 0.18, 0.41, 0.60 respectively and highest Rf value was the 0.60 while the highest height of the peak was for the first peak which is 239.5 Au (Figure 8 and Table 6).

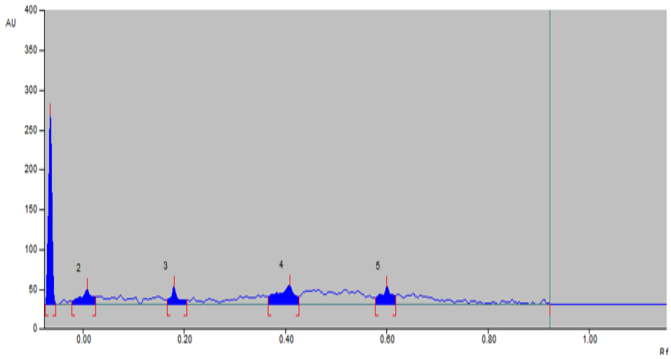

Fig 8: HPTLC fingerprint profiles under 254nm of TM in ratio of 1:1:1

In the ratio of the 1:2:3 showed the 7 peaks with the Rf values of -0.07, 0.08, 0.39, 0.50, 0.67, 0.74, 0.83 respectively and highest Rf value was the 0.83 while the highest height of the peak was for the sixth peak which is 276.0 Au (Figure 9 and Table 7).

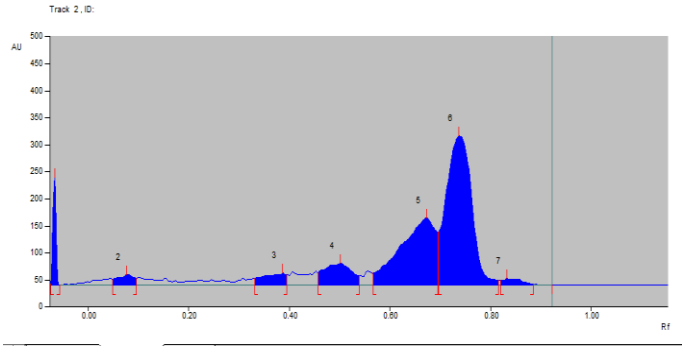

Fig. 9: HPTLC fingerprint profiles under 254nmof TM in ratio of 1:2:3

In the ratio of the 1:2:4 showed the 2 peaks with the Rf values of 0.56, 0.73 respectively and highest Rf value was the 0.73 while the highest height of the peak was for the second peak which is 38.0 Au (Figure 10 and Table 8).

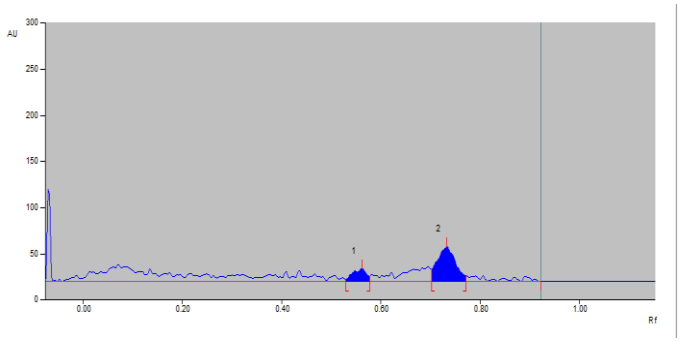

Fig. 10: HPTLC fingerprint profiles under 254nmof TM in ratio of 1:2:4

Pharmacodynamic properties[14,20,21,22]

Pharmacodynamic properties of *Triphala* mentioned in Ayurveda authentic texts were tabulated in Table 9.

According to the findings it showed that common *Guna* of the *Triphala* is *Ruksha* and both *Harithaki* and *Vibhithaka* are *Ushna veerya* and all three are in *Madhura vipaka*. When considering to the pacification of *Dosha*, only *Vibhithaka* is showing *Kapha, Pitha shamaka*.

Discussion

Oral health is identified as an important part of quality of life, and maintaining proper oral hygiene is essential for both the body and oral health. Oral diseases can influence a person's growth and development, as well as their psychic productivity and social capacity. [23]

In *Ayurveda*, *Gandusha* is advised for maintaining oral health. It increases mechanical pressure inside the oral cavity, activates the salivary glands, enhances vascular permeability, and keeps the oral pH stable.

A mouthwash is a solution used to rinse the oral cavity, and it helps oral hygiene by decreasing plaque and tartar or treating periodontal diseases, interdental bleeding, bad breath, and gingivitis.

Table 6: Peak values of HPTLC fingerprint profiles of TM in ratio of 1:1:1

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.08 Rf	0.6 AU	-0.07 Rf	239.5 AU	73.25 %	-0.06 Rf	0.9 AU	1232.6 AU	44.90 %	unknown *
2	-0.02 Rf	4.3 AU	0.01 Rf	19.2 AU	5.88 %	0.02 Rf	9.5 AU	323.3 AU	11.78 %	unknown *
3	0.17 Rf	6.2 AU	0.18 Rf	22.3 AU	6.81 %	0.20 Rf	4.9 AU	232.3 AU	8.46 %	unknown *
4	0.36 Rf	10.0 AU	0.41 Rf	23.6 AU	7.20 %	0.43 Rf	9.2 AU	603.6 AU	21.99 %	unknown *
5	0.58 Rf	8.3 AU	0.60 Rf	22.5 AU	6.87 %	0.62 Rf	10.8 AU	353.5 AU	12.88 %	unknown *

Table 7: HPTLC fingerprint profiles with peak values of TM in ratio of 1:2:3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.08 Rf	2.3 AU	-0.07 Rf	199.7 AU	28.83 %	-0.06 Rf	0.6 AU	924.8 AU	4.37 %	unknown *
2	0.05 Rf	11.2 AU	0.08 Rf	19.2 AU	2.78 %	0.10 Rf	12.6 AU	474.0 AU	2.24 %	unknown *
3	0.33 Rf	12.5 AU	0.39 Rf	22.0 AU	3.17 %	0.39 Rf	18.9 AU	728.0 AU	3.44 %	unknown *
4	0.46 Rf	23.3 AU	0.50 Rf	40.0 AU	5.77 %	0.54 Rf	16.8 AU	1655.7 AU	7.83 %	unknown *
5	0.57 Rf	21.9 AU	0.67 Rf	123.8 AU	17.86 %	0.70 Rf	98.0 AU	6506.2 AU	30.78 %	unknown *
6	0.70 Rf	98.4 AU	0.74 Rf	276.0 AU	39.84 %	0.82 Rf	8.1 AU	10513.1 AU	49.73 %	unknown *
7	0.82 Rf	8.7 AU	0.83 Rf	12.2 AU	1.75 %	0.88 Rf	1.2 AU	339.2 AU	1.60 %	unknown *

Table 8: HPTLC fingerprint profiles of TM in ratio of 1:2:4

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.53 Rf	2.1 AU	0.56 Rf	13.7 AU	26.58 %	0.58 Rf	5.5 AU	285.8 AU	21.70 %	unknown *
2	0.70 Rf	13.1 AU	0.73 Rf	38.0 AU	73.42 %	0.77 Rf	6.2 AU	1031.1 AU	78.30 %	unknown *

Table 9: Pharmacodynamic properties of *Triphala*

Name	*Rasa*	*Guna*	*Virya*	*Vipaka*	*Dosha karma*
Haritaki	*Pancharasa(except lavana),Kashaya pradhana*	*Laghu Ruksha*	*Ushna*	*Madhura*	*Tridoshahara*
Vibhitaki	*Kashaya*	*Laghu Ruksha*	*Ushna*	*Madhura*	*Kapha Pitta Shamaka*
Amalaki	*Pancharasa (except lavana), Amla pradhana*	*Guru Ruksha*	*Sheeta*	*Madhura*	*Tridoshahara*
Triphala	*Kashaya*	*Laghu Ruksha Vishada*	*Anushna*	*Madhura*	*Kapha Pitha Shamaka*

According to *Sharangadara Samhita*, *Triphala* can be used as a *Gandusha* for dental diseases. *Triphala* is one of the well-known polyherbal formulations being used in traditional *Ayurveda* medicine since ancient times. *Triphala* possesses astringent action and anti-bacterial, anti-septic, and anti-inflammatory properties. Because of these properties, it is commonly used as a mouthwash in *Ayurveda*.

In the present study, *Triphala* mouth wash was prepared in the form of *Kwatha* (decoctions) as modification of the dosage form of *Gandusha*. The three myrobalans that are used in the making of *Triphala Kwatha* can be in equal or unequal proportions. Therefore, the present study, *Triphala* mouthwash, was prepared in those three proportions. One is in equal proportions: *Terminalia chebula*, *Terminalia bellerica*, and *Phyllanthus emblica*. The other two are 1:2:3 proportion and 1:2:4 proportion respective to the above-mentioned medicinal plants.

Herbal drug standardization is confirmation of its identity and determination of its quality and purity. It is a tool for quality control. WHO mentioned the specific guidelines for the evaluation of the safety, efficacy, and quality of herbal drugs. Three prepared proportions of *Triphala* mouthwash were analyzed to establish standard parameters. When considering the organoleptic characters, even though 1:2:4 contained more *Amalaki* and it had *Amla Pradhana* in taste during the preparation of decoction, the dominant *Amla* taste might be subsided. However, the *Tiktha kashaya* taste is a bit more in TM2 than in the other two proportions, which may be due to the high proportions of *Vibhithaka* compared with the 1:1:1 ratio and in the comparatively less proportion in the 1:2:4 ratio. *Kwatha* must have a pH between 3 and 5 for better pharmacological action. Analysis showed the pH values of TM1, TM2, and TM3 were 3.98, 3.92, and 3.96 respectively, which are the required pH levels for better effectiveness. The pH of all prepared samples is acidic, which might be helpful in reducing the *Malabhutha Kapha* and thereby reducing the *Mukha Daurgandhatha*. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index according to the WHO guidelines. The height of froth measured was less than 1 cm in every test tube for all three proportions. Provided the mentioned reading for the foaming index.

Qualitative phytochemical analysis was done to detect and compare the chemical constituents of each proportion of *Triphala* mouth wash. The presence of tannin, saponin, phenol, carbohydrate, flavanoid, and alkaloid would be responsible for anti-oxidant, anti-inflammatory, anti-bacterial, wound healing, etc. These drug actions are important in curing lots of diseases that are related to the mouth, such as *Krimi Dantha*, *Mukha Paka*, *Mukha Daurgadha*, etc. *Triphala* has significant antimicrobial action against gram-positive and gram-negative bacteria, namely Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. This is due to the presence of various chemical constituents, like flavonoids and alkaloids. TLC is the initial step to recognizing the phytochemical compounds present in the sample. With the advancement of TLC, HPTLC can provide an electronic image of chromatographic finger prints. In the present study, as we prepared the decoction, the dichloromethane fractions were used to develop TLC and HPTLC fingerprints. Because of the gallic acid is the biomarker for the *Harithaki* and tannin is the biomarker for the *Vibhithaki*, the TLC was developed equivalent to gallic acid and tannic acid. The fingerprint profile of *Triphala* mouthwash in different proportions proved the presence of various bioactive compounds in the decoctions, and tannin and gallic acid could be clearly identified with the markers visualized under UV at 254 and 366 nm. Gallic acid and tannic acid also have anti-oxidant, anti-inflammatory, anti-bacterial, etc. Because of tannic acid and gallic acid, *Triphala* can inhibit Streptococcus mutans, which causes dental plaque and gingival inflammation. Also, *Triphala* shows a significant inhibitory effect on Candida, probably due to gallic acid. A HPTLC study was done to compare the area and intensity of the spots that appeared in the TLC profile. According to the HPTLC analysis, *Triphala* mouth

wash prepared in a ratio of 1:2:3 showed the highest Rf value, which was 0.83.

The strong antioxidant action of *Triphala* may be *Haritaki* and *Vibhithaki*, which are effective for bleeding gums, gingival ulcers, and carious teeth, and *Amalaki* consists of vitamin C, which is most effective to prevent bleeding from gums.

Kapha dosha naturally dominates the oral cavity. *Vikrita kapha*, which is considered *Mala*, is mainly involved in the disease of the oral cavity. The majority of *Mukha roga* are *Kaph,a Raktha pradhana*. Hence, it is effective to use medicines, which are mainly in *Kapha* and *Pittahara* properties. *Triphala* mainly consists of *Kashaya rasa*, and *Kashaya rasa* mainly has *Stambhana*, *Ropana*, *Shodhana*, and *Kaphahara* properties. According to the *Panchamahabhautika* constitution, *Triphala* has *Ruksha*, *laghu* and *Vishadha guna*. These properties are just opposite the *Kapha guna*. Also, *Thriphala* consists of *Katu* and *Tikta rasa*. *Tikta rasa* pacifies the *Pitta*, and *Katu rasa* has the property of *Vaktra shoshana*. Therefore, *Triphala gandusha* is very effective for various kinds of *Mukha Roga*. [24,25,26,27,28,29,30,31,32,33]

The effectiveness of the therapeutic action may vary depending on the proportions of the ingredients. An equal proportion of *Triphala* has *Kapha-Pitta shamaka* property. *Terminalia chebula* and *Phylanthus emblica* consist of *Tridoshahara* property, and *Terminalia bellerica* consists of *Kapha*, *Pittahara* property. Therefore, 1:2:3 and 1:2:4 proportions of *Triphala* mouth washes are more towards the *Kapha*, *Pittahara* property than equal proportion. Hence, 1:2:3 and 1:2:4 proportions may be more effective for *Kapha*, *Raktha pradhana Mukha rogs* than an equal proportion of *Triphala*.

Conclusion

Triphala mouth wash in all three proportions can prevent and heal oral diseases by increasing mechanical pressure inside the oral cavity, stimulating salivary glands, maintaining oral pH, and its anti-oxidant, anti-inflammatory, anti-bacterial, and wound-healing qualities. Hence, all three proportions of *Triphala* mouth wash can be used for oral diseases, but the effectiveness of the therapeutic action may vary slightly depending on the proportions of the decoction. Therefore, 1:2:3 and 1:2:4 proportions may be more towards the *Kapha-Pitta Hara* property than equal proportions.

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Therapeutic potential of *Thabasir* in Sri Lankan Indigenous Medicine: A scientific review

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Abstract

Thabasir (Bamboo salt) is a white silica exudation found in the internodes of stems of the female bamboo (*Bambusa arundinacea*). This concrete like crystalline is an opaque, white, irregular shaped, light, soft and brittle substance. It contains 90 % silica as a hydrate of silicic acid, peroxide of iron, potash, lime, aluminium and vegetable matter. Traditionally it has been used in various ailments such as hyperdipsia, diarrhoea, vomiting, heart diseases, cough, asthma, jaundice, fever, tuberculosis, bronchitis, leprosy, paralytic complaints, anaemia and as a general tonic in convalescents. Further, in recent years scientists have shown more interest in *Thabasir* due to its medicinal, nutritional and cosmetic values. However, up to now, no research studies have been carried out to prove its therapeutic effects scientifically, as mentioned in Unani medicine. Therefore, the information available in this review would help to do further research in this regard. Hence, this review aims to explore the information available in the literature regarding therapeutic potential of *Thabasir* in the field of indigenous medicine. All the available information on *Thabasir* was compiled from search engines of electronic databases such as Google scholar, PubMed, Medline, Scopus and classical texts. The literature search revealed that *Thabasir* possess pharmacological properties such as cardiac exhilarant, cardiac tonic, astringent, cooling, dessicant, febrifuge, general tonic, sexual tonic, tissue builder, aphrodisiac, spermopiotic, thirst quencher, hemostatic, expectorant, diuretic and general tonic. It can be concluded that *Thabasir* is a potential therapeutic agent in Sri Lankan indigenous medicine.

Keywords: *Bambusa arundinacea*; Bamboo salt; *Thabasir*; Therapeutic activity, Traditional medicine

Introduction

Man has continually investigated plants, animals and minerals in order to assess the importance of developing natural, sustainable and affordable drugs for treating various ailments. Among those three sources, the plants of tropical and subtropical origin have been found to have therapeutic potential and are being used since time immemorial. The beneficial therapeutic effects of these medicinal herbs are due to the chemical components present in them. As such, Bamboo is one of the precious plant resources of the earth which plays an important role in indigenous systems of medicine due to its rich nutritional, medicinal and cosmetic values. This review aimed to gather information available in the literature regarding the therapeutic potential of *Thabasir* in the field of indigenous medicine.

Methodology

All the available information on *Thabasir* was compiled from electronic databases of Google scholar, PubMed, Medline, Scopus and classical texts.

Results and Discussion

Scientific classification of Bamboo

Kingdom: Plantae
(unranked): Angiosperms
(unranked): Monocots
(unranked): Commelinids
Order: Poales
Family: Poaceae
Subfamily: Bambusoideae
Supertribe: Bambusodae[1]

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English Name: Bamboo salt; Tamil Name: *Moongil uppu;* Sinhala Name: *Una kapuru;* Tibbi Name: *Tabashir;* Arabic Name: *Tabashir;* Sanskrit Name: *Bansarochana, Vanshalochana, Vamsarocana, Bangsolochan*; Other synonyms: *Subha, Subhra, Tuga, Tugaksiri, Tvakaksiri, Vaisnavi, Vamsaja, Vamsaksiri, Vamsi* [2]

About half of these species grow in Asia, most of them within the Indo-Burmese region which includes 136 species under 23 genera which are available only in India[3]. Most of the bamboos grow in a warm climate, abundant moisture, and productive soil, although some do grow in reasonably cold weather about 20 °C. They grow in plains, hilly and high-altitude mountainous regions, and in most kinds of soils, except alkaline soils, desert, and marsh.

As sugar cane, corn and other grasses, bamboos comprise one of 12 subfamilies within the family Graminae (Poaceae) and they represent the only major grass lineage to diversify in forests. They are distinguished from the other members of the grass family by the presence of branches at each node and well-developed, asymmetrically strongly invaginated arm cells in the leaf mesophyll as seen in cross section and also generally exhibit relatively broad, pseudopetiolate leaf blades usually with fusoid cells flanking the vascular bundles and by the presence of branches at each node. Nearly 1,500 described species of bamboos are classified into three tribes: Arundinarieae (temperate woody bamboos, 546 species), Bambuseae (tropical woody bamboos, 812 species), and Olyreae (herbaceous bamboos, 124 species)[3,4].

A bamboo culm consists of an internode (which is hollow for most bamboo) and a node, which is solid and provides structural integrity for the plant[4]. *Thabasir* is a white silica exudate found in the internodes of stems of the female bamboo. This siliceous concrete is a crystalline, opaque, irregular shaped, light, soft and brittle substance. It contains 70% of silica or silicon as hydrate of silicic acid, peroxide of iron, potash, lime and alumina. Traditionally it has been used in wide range of ailments such as hyperdipsia, diarrhoea, vomiting, heart diseases, cough, asthma, jaundice, fever, tuberculosis, bronchitis, leprosy, paralytic complaints, anemia and as a general tonic in convalescents. Generally, a small quantity of *Thabasir* is available in the bottom and sides of the cavity of bamboo. Therefore, the genuine *Thabasir* obtained from the bamboo culm is adulterated with some other stones such as Calcium carbonate and sold at Sri Lankan and Indian markets.

As per Unani classical texts, *Thabasir* is the limestone which accumulates in the cavity of the female bamboo plant. It is in the form of moisture and it becomes dry later. Its *Mizaj* (Temperament) is *Sard* 1 Vo *Khusk* 2 (cold 1 dry 2). It possesses properties like, *Mufarrih e Qalb* (cardiac exhilarant), *Qabis* (astringent), *mubarrid* (Cooling), *mujaffif* (Desiccant) and *Daf e Humma* (febrifuge). It is mentioned as having adverse effects for sexual organs and lungs and to rectify the adverse effects, it is recommended to use with Honey/ *Pistacia lentiscus* or *Ziziphus jujuba* Mill. Its substitute is *Portulaca oleracea* Linn. and its dose is 1-3 grams. It is mentioned in Unani Medicine that *Thabasir* is beneficial in palpitation of heart, weakness of heart, fever and to quench the thirst. The compound medicines prepared by using *Thabasir* in Unani medicine are *Safoof e Thabasir, Habb e Thabasir, Qurs e Thabasir, Safoof e Sat e Gilo, Qurs-e-Thabasir Mulaiyinin, Qurs-e-Thabasir Qabiz, Jawarish-e-Thabasir* [5,6,7].

In Ayurvedic systems of medicine, two types of bamboo are distributed all over the world. They are: *Bambusa arundinacea* Retz. (Big size) and *Dandrocalamus strictus* (Small size). *Bansalochana* or Manna is found in the interior of the stem of *Bambusa arundinacea,* near the nodes. The camphor of Vansa (*Bambusa arundinacea*) silicious matter found near the joints inside is a white camphor like substance[8]. It is a white silicon concrete crystalline substance which is an opaque, irregular shaped, light, soft and brittle. It contains 90 % silica or silicon as hydrate of silicic acid, peroxide of iron, potash, lime, aluminium, vegetable matter, cholin, betain, nuclease, urease, proteolytic enzyme, diastatic and emulsifying enzyme, cyanogenetic

glucoside[2,9]. Its potency is cold and the taste is sweet. It is a valuable drug which possess *Vata-pittashamana* (neutralizing black bile and choleric humours), *Trishnanigrahana* (reducing excessive thirst), *Grahi* (absorbing excessive moisture of GIT, *Hridya* (Cardiac tonic), *Rakta stambhana* (hemostatic), *Kaphanissaraka* (expectorant), *Shvasahara* (relieving bronchial asthma), *Mutrala* (diuretic), *Jvara-ghna* (febrifuge) and *Balya* (improving strength)[9,10,11].

Therefore, it is useful in the management of ailments such as hyperdipsia, diarrhoea, vomiting, heart diseases, cough, asthma, jaundice, fever, tuberculosis, bronchiectasis, lung cavities, bronchitis, leprosy, paralytic complaints, anaemia, emaciation and as a general tonic in convalescents. Generally, it is very difficult to get the genuine *Vansalochana* as it is to be obtained from bamboos which are to be split open [8][12].

Conclusion

The literature reveals that the various therapeutic properties of *Thabasir* mentioned in Unani and Ayurvedic medicine such as cardiac exhilarant and tonic, astringent, cooling, desiccant, febrifuge, tissue builder, aphrodisiac, spermopiotic, thirst quencher, haemostatic, expectorant, diuretic and as a general tonic. However, up to now no research studies have been carried out to prove these therapeutic effects scientifically. Therefore, the information available in this review would help to do further research in this regard. Further, due to its cost and difficulty in getting the genuine sample, adulteration is very common with *Thabasir*.

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A Review on Unani Management of *Amraz e Qalb*

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Abstract

One of the body's essential organs is the heart, also referred to as *Qalb*. The Unani system of medicine defines the cardiac diseases under *Amraz e Qalb.* All over the world, cardiovascular diseases (CVDs) account for the foremost cause of mortality. Regardless of developing and non-developing countries CVDs cause a greater burden of disease**.** Unani scholars gave the utmost important to every facet of the treatment of *Amraz e Qalb*. Effective principles of treatment to be followed have been recorded along with effective *Mufrad vo Murakkab Advia* (Single and compound medicines), *Ilaj bil Tadbeer* (Regimenal therapies) and, *Hidayat* (Advice). The study is aimed at compiling those effective regimes paying particular attention to the drugs that have shown potent cardioprotective activity. Authentic Unani text books, and pharmacopoeias were reviewed to get information on *Amraz e Qalb* and its management. A search of scientific journals and research articles was conducted to determine the cardioprotective activity of the commonly given medications. There are sufficient natural medications to adequately treat CVDs. While more scientific research needs to be conducted in the future, this study has provided a significant quantity of information on the management of *Amraz e Qalb* that might be used for the successful treatment of CVDs.

Keywords: *Amraz e Qalb*, Cardiovascular diseases, Cardioprotective activity

Introduction

The heart, also known as *Qalb* is a vital organ of the body. The Unani system of medicine defines the cardiac diseases under *Amraz e Qalb*. The modern medicine describes cardiovascular diseases (CVDs), encompassing coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism. All over the world, CVDs account for the foremost cause of mortality. 17.9 million of people died of CVDs in the year 2019, hence 32% of global deaths were attributed to CVDs. Heart attacks and strokes were the cause of 85% of these fatalities. Regardless of developing and non-developing countries CVDs cause a greater burden of disease. More than 75% of deaths from CVDs are recorded in the low- and middle-income countries[1]. Assuring that in Sri Lanka, 2.6% of total deaths account for coronary artery diseases, and also being placed as the leading cause of death, while stroke is ranked as the fifth leading cause of death. The second most cause of death and a significant risk factor for the CVDs in Sri Lanka is diabetes mellitus.[2]

It was found that unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol are the commonest behavioral risk factors that lay a path to CVDs by inducing the intermediate risk factors of CVDs such high blood pressure, high blood glucose level and raised blood lipids and overweight and obesity. Hence the modern world suggests, eating a healthy balanced diet with low salt, sugar and fat, regular exercise, cessation of smoking, reducing or abstaining from alcohol use help to prevent the CVDs. Further, the early detection and appropriate treatment would reduce the incidence of deaths due to CVDs.[1,3] In line with this, Unani scholars have described an extensive way of management of

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Amraz e Qalb in the antiquated literatures decades ago, involving the preventive and curative aspect including almost all the treatment modalities of Unani medicine such *Ilaj bil Ghiza* (Dietotherapy), *Ilaj Bil Dawa* (Drug therapy), and *Ilaj bil Tadbeer* (Regimenal therapy). Unveiling those treatment packages would be a greater contribution to overcome the disease burden of CVDs. Avicenna, the greater Unani physician wrote a complete book on the medicines for cardiac diseases called, *Kitab al Advia al Qalbia*. Hence, the study is aimed to gather efficient medications as well as alternative forms of care, paying particular attention to those medications that have been shown to have a cardioprotective impact in order to effectively manage CVDs in the future. Therefore, objectives of this review were to find out effective medicines for the management of *Amraz e Qalb* mentioned in Classical Unani texts and to bring out the effective principles of treatment and treatment packages in the management of *Amraz e Qalb*.

Methodology

Renown classical books of Unani Medicine such as, *Al Qanoon fil Tib*, *Tarjuma e Kabeer*, *Adviya Qalbiya*, *Al Havi Al Kabeer*, *Trajuma e Kamil us Sanah*, *Moalejat e Boqratiya*, and *Qarabadeen* etc. were reviewed to get information on *Amraz e Qalb* and its management. The collected data is scrutinized to get the information on single drugs, compound preparations, Regimenal therapies and advices that could be used for the effective management of heart diseases. Further, most valuable and available drugs were filtered out of huge lists of drugs with great effort. Out of the all cardiac drugs, few, special, known to be effective and commonly used in many prescriptions were selected and analyzed for their cardioprotective effect.

Results and Discussion

Types of Amraz e Qalb

A wide range of cardiac diseases and specific symptoms related to the cardiac diseases have been mentioned by the Unani scholars. List of the cardiac diseases discussed in the classical Unani texts are as below,

Soo e Mizaj Qalb temperament of Heart	- Pathological
Khafqan	- Palpitation
Waja ul Qalb	- Chest pain
Ghashi	- Syncope
Zaghtul Qalb heart	- Black bile filled in
Istisqa ul Qalb	- Congestion of Heart
Zoaf e Qalb	- Weakness of Heart
Qazful Qalb coming out	- Feeling of heart
Jazb ul Qalb	- Descent
Taqashshurul Qalb	- Maceration of Heart
Amraz Samamat e Qalb diseases [4,5,6,7]	- Valvular Heart

Unani medicine, greatly deals with heart disorders. *Soo e Mizaj Qalb* (Heart pathological temperament), *Khafkhan* (Palpitation), and *Waj ul Qalb* (Chest pain) have been extensively discussed. Therefore, the management of these three disorders is the primary emphasis of the study.

Soo e Mizaj Qalb - Pathological temperament of heart

Soo e Mizaj Qalb is primarily due to *Shadeed Haar vo Baarid* (Intense heat or cold). *Quwwat e Haiwani* (Vital power) and *Qalb* are hot in temperament, when the temperament changes towards *Soo e Mizaj Haar* (Intense heat mal temperament), metabolism of heart is increased. Aim of *Usool e Ilaj* (Principles of treatment), is to reverse the normal temperament of heart by giving cold drugs internally and externally. However, cold drugs alone are not advisable, and cold drugs should be accompanied by some hot drugs to preserve internal heat of the *Qalb*. Internally, *Sharbat e Anar* (Syrup of Pomegranate) and *Sharbat e Sandal* (Syrup of Sandal wood) could be given. While externally cold temperamental *Zimad* (Poultice) made of *Kafoor* (Camphor), *Sandal safed* (White sandalwood), *Gulab* (Rose), *Tabasheer* (Bamboo salt), *Kashneez* (Coriander) also to be applied over the cardiac region.[5]

Intense cold atmosphere could affect the heart and cause death at times. When the temperament shifts to *Soo e Mizaj Barid* (Intense cold mal temperament), internally single drugs of hot temperament such as *Darunuj Aqrabi* (Doronicum), *Jadvar* (Zedoary), *Musk* (Musk), *Ambar* (Ambergris), *Zaranbad* (Long zedoary), *Abresham* (Silk coccon), *Sumbul* (Nard) could be given. Further compound preparations like, *Dawa ul Misk, Mufarrih e Har, Sharbat e Gaozaban, Sharbat e badranjboya* and *Sharbat e Ood* could also be prescribed. *Garam vo Khushbudar Zimad* (Hot and dry poultice) made of *Sunbul, Nagarmota, Darcheeni* (Cinnamon), *Gul Surkh, Lavang* (Clove), *Aab e Marzanjosh* (Water of Oregano), *Aab e Badranjboya* (Water of balm mint) and *Aab e Reehan* (Basil water) to be applied on the chest. Patient should be advised to have *Maghziyat* **(**Nuts) and *Garam Ghiza* (Hot diet) like *Gosth* (Young birds' flesh) cooked with spices *Darcheeni, Zafran* (Saffron), *Zeera* (Cumin). Added to that moderate exercise is prescribed. [4,5,6,8,10]

Muqavviate Qalb (Cardiac Tonic) and *Mufarrih Qalb* (Exhilarants) to be given to strengthen the heart and to regulate the functions, while correcting the imbalance of temperament in both conditions. *Muqavviate Qalb vo Mufarrih Qalb Haar and Barid* should be used in *Soo e Mizaj Haar Qalb* and *Soo e Mizaj Barid Qalb* respectively.[4,5,6,10] A list of *Murakkab Dawa* (Compound medicines) of *Muqavviate Qalb* and *Mufarrih Qalb* mentioned in the classical texts are mentioned in Table 1.[4,5,6,7,8,9,10,11,12]

Apart from *Soo e Mizaj Haar* and *Soo e Mizaj Baarid*, temperament could deviate to *Soo e Mizaj Ratab* (Moist mal temperament) and *Soo e Mizaj Yabis* (Dry mal temperament). However, these abnormal temperamental changes need not special medicines as heart will not be affected as abnormal hot or cold temperament. In *Soo e Mizaj Yabis*, large quantities of moist foods to be given, for instance *Ma us Shaeer* (Barley water) with *Roghan e Badam* (Almond oil) is beneficial. Baths are given or the body is sponged after food. Patient feels much better after long sleep which should be encouraged. Patient would be enjoyed having a large quantity of cold water but it should be prohibited if the patient has *Zukam* (Cold). Further, bodily movement and exercise to be restricted. Drugs which produce dryness are useful in *Soo e Mizaj Yabis* like *Lavang, Zafran, Badranjboya* and strong alcohols. Patient should be advised to bath before meal and to do moderate exercise.

Table 1: A list of *Murakkab Dawa* (Compound medicines) of *Muqavviate Qalb* and *Mufarrih Qalb* mentioned in the classical texts

Muqavviate Qalb vo Mufarrih Qalb Haar	***Muqavviate Qalb vo Mufarrih Qalb***
Mufarrih Haar	*Mufarrih Barid*
Dawa ul Mishk	*Khameer e Sandal Sada*
Dawa ul Kurkum	*Khameer e Marwareed*
Khameer e Abresham Sada	*Khameer e Khas*
Khameer e Gaozaban Sada	Sharbat e Anar
Sharbat e Badranjboya	Sharbat e Seyb
Sharbat e Gaozaban	Sharbat e Behi
Sharbat e Ood	Sharbat e Nilufar
Majun Lana	Sharbat e Gul e Gudal
	Sharbat e Vard

Khafkhan - Palpitation

Khafqan / Ikhtilaj is a condition where heart pumps vigorously that the patient feels the contraction which is not normally felt. *Khafkhan* may arise due to several causes. *Khafkhan* due to *Ghair e Tab' aee Akhlat* (Morbid humour) that appears as a result of qualitative and quantitative changes in the humoural matters, should be corrected according to the involved morbid *Akhlat.* In *Imtela e Aweya*, especially, the quantity of *Khoon* is raised where increased congestion of the blood vessels contracts the heart more. In this condition *Fasd* (Venesection) on the Basilic vein is beneficial and *Aqras e Kafoor* (Pills of Camphor) could be prescribed.[5]

Heart gets intoxicated when *Ghair e Taba' ee Sawda* (Offending black humour) reaches it.

Khafkhan appears as a protective mechanism when *Tabee'at* tries to overcome this intoxication. This condition should be corrected by expelling the offending *Sawdavi Madda* using, *Sawdavi* purgatives like *Ayarij e Lughazia* and *Ayarij* e *Rufas*. *Haleel e Siyah* (Chebulic Myrobalan), *Aftimoon* (Dodder), *Hajar e Armani* (Bole Armania) mixed with *Dawaul Misk* and *Sharbat e Reehan* could be prescribed. *Aftimoon* and *Ayarij Feeqra* should be continuously taken with *Sikanjabeen* (A mixture of vinegar and honey). This condition should be treated as *Malikholia* (Melancholia). Drugs to strengthen heart should also be accompanied.[5]

Heart tries to maintain its natural innate heat by increasing its function, when *Ghair Taba'ee Bulghum* (Offending white humour) affects the heart, as a result of this heart beat increases. Offending white humour should be expelled. Expectorants which help in the excretion of slim and sticky matter are more useful. Initially, medicines which reduces the viscosity like *Usara e Turb* and *Sikanjabeen* should be given. Later, A *Nuskha* (Prescription) of purgatives, made of *Ghariqoon* (White ageric), *Shaham e Hanzal* (Colocynth), *Turbud* (Turpeth), *Muqil* (Guggul), *Ood e Hindi*, *Musk*, *Zafran* and *Nafti Namak* could be prescribed. *Khafkhan* due to excessive *Safra* is rare. However, when *Safra* is the offending humour offer *Rubub ul Fawaqia* (Extracts of citrus fruit) and sweet-smelling fruits such as *Safargel* (Behi), *Amrud* (Guava), *Seib* (Apple) after meals.[5]

Qualitative and quantitative changes of the blood in *Faqruddum* (Anaemia) lead to weakness of the heart, palpitation occurs where heart tries to get rid of this weakness. Drugs to strengthen the heart should be prescribed [8]. Further, *Khafkhan* appears due to the use of *Tambako* (tobacco), *Sharab* (alcohol) and *Bhang* (Cannabis). In this case, the substance used should be withdrawn slowly. Then, *Muqawwiyat e Qalb* drugs should be given to strengthen the heart. *Mufarrih e Barid*, with *Sandal safed, Kashneez khushk, Arq Gaozaban, Arq Kewda* by adding *Sharbat Seyb* or *Sharbat Anar* could be prescribed in the morning, while in the Evening *Zulal* (soaked mucilaginous water) of *Gul e Gudal Sabz* (fresh shoe flower) soaked in *Arq Gulab* (Rose water) and *Arq Gaozaban* by adding *Sharbat Nilufar* (Syrup of Lotus) could be given.[5]

Khafkhan due to *Infialat e Nafsaniya* (Psychological effects) could be treated by prescribing *Murakkab Dawa* such, *Khameer Abresham Hakeem Arshad wala, Khameer Abresham Unab wala, Sharbat Seyb* (Apple Syrup) with *Arq Gaozaban* (Distilled water of Borage), *Mufarrih barid* with *Sharbat Anar* (Syrup Pomegranate) If the patient complains of *Qabz* (Constipation), *Gul Qand* (A confection of Rose) could be added.[4,5,8]

Honey with hot water could be given for the occurrence of *Khafkhan* during pregnancy. Following principles of treatment to be adopted to correct *Khafkhan* developed due to pathologies of other adjacent organs like stomach, liver etc. by doing *Fasd* (venesection) on Basilic vein followed by *Munzij vo Mushil Sawda* (Coction & evacuation of atrabilious*), Muqavvi e Qalb*, thin pleasant smelling *Jawarishat* and *Hamam* (Bath) with pleasant smelling drugs.[4,5,8`]

Waja ul Qalb -Chest pain

Waja ul Qalb (Chest pain) is described as a condition that there will be acute chest pain at the site of heart which may lead to death at times. *Ma'ajun Barsha'asha* could be given as *Musakkin e Alam* (Analgesics) to alleviate pain. Additionally, *Mufrad Dawa* (Single drugs) of *Mufatteh e Sudad* (Deobstruants) such *Darchini, Lehzan* (Garlic), *Zaranbad, Adrak* (Ginger), *Shehad* (Honey), *Kaloonji* (Black cumin), and *Zafran* should be included. *Joshanda* made of *Tukhm Kasoos, Tukhm Badranjboya, Shahatra, Badiyan, Gul e Surkh, Aftimoon, Darunaj Aqrabi, Tukhm Qurtum, Maveez Munaqqa* and *Gul e Gaozaban* with *Gulqand* is a good *Mufatteh e Sudad*. It should also include *Muqavvi e Qalb* drugs like *Sharbat Anar, Sharbat Vard Muqarrar, Jawahar Mohra* and *Khameer e Gaozaban*. Encourage the patient to vomit if they often nauseate by utilizing *Muqayyat* (Emetic drugs). *Hazimat* (Digestives) including *Ilaichi, Gulab, Badiyan, Tabasheer, Kashneez* or *Anushdaru*

must be administered. Fatty diets and spices should be avoided. *Shamumat* (Inhalation of fragrant things) *Mishk, Amber* and *Kafoor* could be inhaled. It is forbidden to engage in either mental or physical exercise. It is beneficial to do *Takmeed* (Fomentation) with aloe on the chest area by sprinkling *Haldi* (Turmeric) and *Sohaga* (Borax) over it.[5,8]

Singles drugs that have been frequently mentioned in the prescriptions of classical Unani texts with special attention to their scientific evidence on Cardioprotective action

***Anar – Punica granatum – Pomegranate* (Figure 1)**

Niewiadomska, J. *et al* (2023) assessed the cardioprotective in Zucker diabetic fatty rats using the pomegranate peel extract by evaluating the oxidative stress markers and biomarkers of heart failure. Study revealed that pomegranate peel extract has anti-free radical effects in the myocardium.[13]

A study by Zahra, R. (2017) to assess the cardio protective effect of *Punica granatum* juice in ischemic heart disease with the conventional therapy revealed that the level of serum troponin and Malondialdehyde were markedly reduced and showed a significant decrease in angina pectoris frequency and intensity in patients with unstable angina.[14]

Fig.1: Anar – *Punica granatum*

***Sandal Safed – Santalum album - White sandalwood* (Figure 2)**

Kamal A, et al. (2022) conducted a study employing isoproterenol to induce myocardial infarction in order to assess the cardioprotective impact of Sandal e Safed in murine model. Two groups of mice that received the dose of 600mg/Kg and 800mg/Kg powder of sandal wood orally showed normal cardiac enzymes, lipid profile and histopathological changes without any side effects.[15]

Fig.2: *Sandal Safed – Santalum album*

Pullaiah, C, P. et al. (2017) looked at the cardioprotective effect of *Rosa damascena* Mill. ethanolic extract on changes in cardiac lysosomal enzyme activity and membrane-bound Na/K/ATPase against isoproterenol induced myocardial infarction in rat models. Ethanolic extracts *Rosa damascena* Mill. significantly stopped the changes and returned the serum Creatine kinase-MB, Lactate dehydrogenase, tissue antioxidants, and lysosomal enzyme activity to almost normal levels in rats.[16]

Another study was designed to assess the cardioprotective effect of ethanolic extract of *Rosa damascena* Mill. against isoproterenol induced myocardial infarction in rat. extract of *Rosa damascena* demonstrated myocardial recovery by restoring cardiac marker enzymes, lowering plasma lipid profiles, and increasing HDL, Additionally, level of malondialdehyde decreased while antioxidant levels in the myocardium rose.[17]

Fig.3: *Gul e Surkh* - *Rosa damascena* - Rose

***Zafran* - *Crocus sativus* – *Saffron* (Figure 4)**

Ghorbanzadeh, V. et al. (2017) investigated the cardioprotective role of crocin; a bioactive substance

in the stigma of saffron along with voluntary exercises in diabetic rats. Crocin and exercise promoted cardiac angiogenesis, perhaps by boosting the expression of endothelial cell specific Mir-126 and Mir-210 which are responsible for neoangiogenesis. Hence, it was found that Crocin and voluntary exercises shown to have protective effect against cardiovascular diseases.[18]

Nader, M. et al (2016) conducted a study to assess the cardio protective effect of saffron supplementation against ischemia reperfusion injuries in rat heart models. Supplementing with saffron decreased myocardial damage and improved cardiac function as the biochemical markers showed reduction in lipid peroxidation and infarct size along with increased antioxidant activity. Further, Electrographic findings showed a significant decrease in both premature ventricular contraction and ventricular tachycardia/ fibrillation in comparison with ischemia reperfusion hearts.[19]

Fig. 4: *Zafran - Crocus sativus*

***Darcheeni - Cinnamomom zeylanicum – Cinnamon* (Figure 5)**

Elmongy, N. et al (2022) investigated for the cardioprotective effect of aqueous extract of cinnamon bark in high fat and high fructose diet fat rats. The current study showed that, combination therapy with pioglitazone and cinnamon extract significantly ameliorated the cardiomyopathy caused by the high fat and high fructose diet possibly by the antioxidant, anti-inflammatory and hypolipidemic mechanisms in the experimental rats.[20]

Jayasinghe, A.N. S. et al. (2021) carried out a in vivo study to assess the cardio protective efficacy of *Cinnamomum zeylanicum* bark extract against doxorubicin induced cardiotoxicity. Total antioxidant capacity, reduced glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase activity were significantly lower than the doxorubicin control group. Hence, it was revealed that, *Cinnamomum zeylanicum* bark extract showed a significant cardioprotective activity through its anti-oxidant and anti-inflammatory potency.[21]

Fig. 5: *Darcheeni - Cinnamomom zeylanicum*

***Zaranbad – Curcuma zedoaria – White Zedoary* (Figure 6)**

A recent study by Amrullah, A. (2021) was carried out on ethanolic extract of *Curcuma zedoaria* to assess the cardio protective activity against cyclophosphamide induced cardiovascular complications in rats. Catechin was used as a positive control. Creatine kinase MB and serum troponin level were come down along with the return of normal histopathology after treatment with increasing dose of ethanolic extract of *Curcuma zedaoria* matching the results of catechin treated group, proved the cardioprotective efficacy of *Curcuma zedoaria*.[22]

Fig. 6: *Zaranbad – Curcuma zedoaria*

***Abresham – Bombyx mori – Silk Cocoon* (Figure 7)**

Srivastav, R. (2022) investigated the cardioprotective potency of *Bombyx mori* against isoprenaline induced myocardial infarction in rats. Pre-treatment with the ethanolic extract of *Bombyx mori* (EEB) in the myocardial infarction induced

rats, especially the high dose of EEB, showed restoration of cardiac histopathology and biochemical markers such alanine transaminase, aspartate transaminase, creatine kinase lactate dehydrogenase and troponin-I.[23]

Fig. 7: *Abresham – Bombix mori*

***Badranjboya - Melissa officinalis – Balm Mint* (Figure 8)**

Nevena, D. et al. (2022) evaluated the cardioprotective activity of ethanolic extract of *Melissa officinalis* (EEMO) via an in vivo study against experimental autoimmune myocarditis rat models. Echocardiographic findings of Ejection fraction, inflammatory markers were significantly improved after the supplementation with EEMO. Specifically, the experimental group that received 200mg/kg of EEMO showed a significant increase in anti-oxidant activity, cardiac performance and myocardial architecture. Hence, it was concluded that Melissa officinalis is a good supportive therapy for autoimmune myocarditis.[24]

Sedighi, M. et al. (2019) investigated the efficacy of ethanolic leaf extract of *Melissa officinalis* as a cardioprotective drugs on ischemia reperfusion injuries in experimental rats. Extract of Melissa officinalis treated group showed a reduction in the size of infarct and episode of arrhythmia compared with that of control group. The mechanism of cardioprotective action is said to be due to the anti-oxidant capacity of the *Melissa officinalis* extract.[25]

Fig. 8: *Badranjboya - Melissa officinalis*

***Gaozaban - Onosma bracteatum – Borage* (Figure 9)**

Strengthening effect of Arq e gaozaban (Distilled water of *Onosma bracteatum*) in heart was investigated through the parameters of cardiac muscle contraction and heart rate in experimental frog heart. Cardiac contraction force increased significantly after employing *arq e gaozaban* with the involvement of calcium channels proving its significant positive inotropic action. It was concluded that due to the myocardial strengthening effect, *Arq e Gaozaban* could be administered in various ailments of the heart.[26]

Fig. 9: *Gaozaban - Onosma Bracteatum*

***Gul e Gudhal - Hibiscus rosa sinensis – Hibiscus* (Figure 10)**

An in vivo study to assess the cardioprotective activity of *Hibiscus rosa sinensis* against ischemic reperfusion injury in murine models by Gauthaman, K.K. et al. (2006) revealed that all treatment groups with oral pulverized flower of Hibiscus rosa sinensis were shown significant increase in antioxidant markers, while the 200mg/Kg treated rat group showed a significant reduction in thiobarbituric acid reactive substances/TBARs (lipid peroxidation marker) and recovering histopathological structure of the heart.[27]

Fig. 10: *Gul e Gudhal - Hibiscus rosa sinensis*

***Angoor - Vitis vinifera- Grapes* (Figure 11)**

Sergazy, S. et al (2020) conducted a study to assess the cardioprotective effect of grape phenol extract against doxorubicin induced cardiotoxicity in rat models. Administration of grape phenol extract showed an increase in antioxidant activity through increased level of superoxide dismutase, catalase, and glutathione peroxidase. Reversal of microscopic myocardial damages to normal was also noted. Hence, it was concluded that grape phenol extract is a good source for the management of heart diseases.[28]

Another study by Razmaraii, N. et. (2016) in doxorubicin induced cardiotoxicity to determine the protective effect of grape seed extract in the heart of experimental rat models revealed improvement in the echocardiographic parameters such ejection fraction and fraction shortening and ECG findings and Myocardial structures were also returned to normal after treatment with GSE, through the anti-inflammatory and anti-oxidant properties.[29]

Fig. 11: *Angoor - Vitis vinifera*

***Seib - Malus domestica – Apple* (Figure 12)**

Rukshana, N. (2022) assessed cardioprotective efficacy of ethanolic extract of *Malus domestica* in experimental rat models. Increasing levels of glutathione peroxidase and superoxide dismutase enzymes at high doses of extract of *Malus domestica* showed a significant antioxidant activity, while the total antioxidant capacity was not substantial compared with vitamin c. Extract of *Malus domestica* against Arachnoid acid and platelet activating factor platelet induced platelet aggregation, showed a noteworthy anti platelet activity. Hence, it was suggested that antioxidant potency, antiplatelet and calcium channel activities of ethanolic extract of *Malus domestica* were the key paths to exert its cardioprotective mechanism.[30]

A comparative study on the cardioprotective effect of phenols of apple peel and apple flesh against high fat and high fructose diet fed mice discovered that both peel and flesh of the apple has significant cardioprotective efficacy. However, peel of apple demonstrated a potent cardioprotective activity as it possesses considerably high amount of total phenols and total flavonoids than flesh of apple.[31]

Fig. 12: *Seib - Malus domestica*

Conclusion

Though there is a sophisticated medical system, a hike in the prevalence of CVDs along with increased disease burden is a huge problem nowadays. Hence, this study would be a valuable source to overcome CVDs and their complication as it reveals the knowledge on the Unani management of Amraz e Qalb that could be effectively employed to manage cardiac diseases. Unani philosophers have not only prescribed medicines, rather they have given a complete treatment compendium. Further, most of the drugs that have been administered in Amraz e Qalb are proven for their cardioprotective action. Hence, this study has provided a significant quantity of information on the management of Amraz e Qalb that might be used for the successful treatment of CVDs in the future.

Conflict of interest

The authors have no conflict of interest to declare.

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