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Cover story

Kowakka

***Coccinia grandis* Linn.**
Family: CUCURBITACEAE
Vernacular names: Sinhala: *Kowakka;* **Sanskrit:** *Bimbika;* **English:** *Ivy Gourd, Scarlet – fruited Gourd, Scarlet gourd, Tindora, Kowai fruit;* **Tamil:** *Kovval, Kovai;* **Hindi:** *Kundru, Tendli*

The plant shown on the cover page is *Coccinia grandis* Linn. It is a fast-growing climbing and ground-keeping perennial vine with a tuberous root stock, with annual stems up to several meters long. Leaves are arranged alternately along the stems, broadly ovate with a basal sinus. The leaf's upper surface is hairless; the lower surface is hairy. The tendrils are long, elastic with coil-like spring character that can wrap around the host to the entire length[1]. The flowers are largely white and star-shaped. Staminate flowers are solitary, rarely in axillary clusters of 2-3, with pedicels 15 to 50 millimeters long. Fruit changes green to red color when ripen and it is ovoid to elliptical, glabrous, and hairless on stalks. Seeds are tan-colored with thickened margins.

Its native range extends from Africa to Asia including India, Philippines, Cambodia, China, Indonesia, Malaysia, Myanmar, Thailand, Vietnam, Papua New Guinea, and Australia. Invasive in Hawaii and the Marina Island. In Sri Lankan traditional Medicine, *C. grandis* preparation is used for the treatment of Diabetes Mellitus, Urinary tract infections, Bronchitis, ulcers, and itchy skin eruptions. The plant is used as a laxative.

The studies showed that ivy gourd is a good source of proteins, minerals, vitamins, and other phytochemicals such as Polyphenols, Flavonoids, Saponin, Glycoside, Bamyrine, Lupeol, Cucurbitacin, Cephalandrol, Cephalandrine and Sterol[2] contents and medicinal effects such as antioxidant, anti-inflammatory, antidiabetic, antibacterial, Hepatoprotective, anti-ulcer, antihyperlipidemic, antipyretic, anti-cancer and analgesic potential[3].

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Sri Lanka Journal of Indigenous Medicine (SLJIM)

Volume 08 Number 01 Page 667 - 722 June 2023

Contents

Efficacy of *Triphaladi kwatha gandusha, Priyangavadi churna pratisarana* and *Triphala churna* orally in the management of *Shitada* (Gingivitis)- An open labelled randomized comparative clinical trial

Sooriyaarachchi B.S.M.M.[1*] Vaghela D.B.[2] and Pawar D.K.[2]

Abstract

Shitada (Gingivitis), a *Dantamulagataroga* of varying severities is nearly universal. Acharya Sushruta has mentioned including *Gandusha*, a *Pratisarana* in the management of *Shitada*. This study mainly focused to compare the efficacy of *Triphaladi kwatha gandusha, Priyangavadi churna pratisarana* and *Triphala churna* orally in the management of *Shitada* (Gingivitis) and the treatment period is one month. In this clinical trial, 32 patients were registered and all the patients completed the treatment. In Group A, 16 patients had been prescribed *Triphaladi kwatha gandusha, Priyangavadi churna pratisarana* and *Triphala churna* orally and in Group B, 16 patients had been prescribed *Triphaladi kwatha gandusha* and *Priyangavadi churna pratisarana*. The assessment was done on the basis of signs and symptoms i.e. *Akasmata Rakta Srava* (Bleeding), *Shotha* (Inflammation), *Krishnata* (Discoloration of gums), *Mukha daurgandhya* (Halitosis), *Vedana* (Pain), *Dantamamsa prakledata* (Moistness), *Paka* (Pus discharge), *Dantamamsa mriduta* (Sponginess), Gingival Index and Bleeding Index. The results showed that complete remission was observed in 31.25% and 6.25% of patients in groups A and B, respectively. The study concludes that the administration of *Triphaladi kwatha gandusha, Priyangavadi churna pratisarana and Triphala churna* orally is more effective in the management of *Shitada* (Gingivitis).

Keywords: *Shitada, Gandusha, Pratisarana*

Introduction

Shitada is one of *Dantamulagataroga.* Sudden bleeding from gums which are foul-smell, blackish, moistened, softened, and get necrosed and suppurated one after the other are signs and symptoms of *Shitada* caused by the vitiated *Kapha* and *Rakta dosha*[1]. Gingivitis is an inflammation of the marginal, unattached terminal end of gingivae which results mainly from the accumulation of debris, plaque and calculus at the tooth margin[2]. Based on signs and symptoms, *Shitada* can be correlated with Gingivitis in contemporary science. *Shitada* is the possible result that takes place due to the lack of oral hygiene and the adoption of improper hygienic methods. The epidemiological studies conducted by the American Academy of Periodontology show that gingivitis of varying severities is nearly universal and it is estimated that over 80% of the world's population suffers from gingivitis[3]. In India, data from the national oral health survey (2002-2003) states that in children aged 12 years, the prevalence of periodontal disease was 57% and in the 15 years of age group, it was 67.7%. The prevalence was 89.6% and 79.9% in the 35-44 year and the 65–74-year age group respectively[4]. In Gujarat, the prevalence of Gingivitis was found to be 74.45%[5]. Currently, the modern medical management of Gingivitis is not sufficient. Acharya Sushruta has mentioned a *Gandusha* which is in the form of decoction containing *Shunthi* (rhizomes of *Zingiber officinalis* Rosc), *Sarshapa* (seeds of *Brassica campestris*

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Linn.), *Triphala* (fruits of *Terminalia chebula* Retz., *Terminalia bellerica* Roxb, and *Terminalia chebula* Retz.), *Musta* (tubers of *Cyperus rotundus* Linn.) and *Rasanjana* (extracts of *Berberis aristata* Roxb. ex. DC.) in the management of *Shitada.* Also, he advises applying locally (*Pratisarana*), the combination of *Priyangu* (flowers of *Callicarpa macrophylla* Vahl.), *Musta* (tubers of *Cyperus rotundus* Linn) and *Triphala* (fruits of *Terminalia chebula* Retz., *Terminalia bellerica* Roxb, and *Terminalia chebula* Retz.)[6]. *Triphala* represents the combination of *Haritaki, Bhibhitaka* and *Amalaki,* a medicinal preparation being used in Ayurveda since ancient times. *Triphala* possesses the properties of *Pancha rasa* except *Lavana rasa*. *Laghu* and *Ruksha guna, Tridosha Shamaka, Shothahara* and *Rasayana.* It pacifies *Kapha* and *Pitta dosha* and is known to have various therapeutic effects such as anti-inflammatory, anti-caries, antioxidants and anti-microbial activities. Hence in this research work, above mentioned *Triphaladi kwatha gandusha* and *Priyangavadi churna pratisarana* were selected as per the guidance of Sushruta Samhita and *Triphala churna* (orally) was selected according to the properties of the drug for the management of *Shitada*. The aim of this study was to compare the efficacy of *Triphaladi kwatha gandusha, Priyangavadi churna pratisarana* and *Triphala churna* orally in the management of *Shitada* (Gingivitis).

Materials and Methods

Study design

32 patients from the Out-patient Department of *Shalakya Tantra,* I.P.G.T. & R.A. Hospital, who was suffering from the disease *Shitada* (Gingivitis) and fulfilling the inclusion criteria of the present study were registered and divided randomly into two groups. All participants in the study were volunteers and they were informed of the outline, purpose and duration of the study and signed an informed consent form before enrolment. All the patients completed the treatment. A carefully arranged research proforma was specially designed for the purpose of incorporating all aspects of the disease on Ayurvedic and modern aspects. This clinical study was initiated after obtaining clearance from Institutional Ethics Committee under the letter No. PGT/7/-A/Ethics/2019-20/2588, dated 08/01/2020 and the study was registered in the Clinical Trial Registry of India under the CTRI No. CTRI/2020/02/23278 [Registered on: 04/08/2018]. Selected patients were randomly divided into two groups and Informed consent was taken from each patient. Following drug schedule was carried out in each particular group for one month time.

Group A:

16 patients were administered local application of 2gm of *Priyangavadi churna* (for 05 minutes) with bee honey two times per day after meals, 20ml of *triphaladi kwatha gandusha* (continued till the patient develops *Kaphapurnaasayata* (mouth is filled with *Kapha*), *Ghranasrava* (watering with nose) and *Akshisrava* (eyes watering) two times per day after meals and 5gm of *Triphala churna* with lukewarm water two times per day after meals.

Group B:

16 patients were administered local application of 2gm of *Priyangavadi churna* (for 05 minutes) with bee's honey two times per day after meals and 20ml of *Triphaladi kwatha gandusha* (continued till the patient develops *Kaphapurnaasayata, Ghranasrava* and *Akshisrava*) two times per day after meals.

Follow-up period

Follow-up was done after the completion of clinical trial for period of 1 month at the interval of 15 days.

Inclusion Criteria

Patients between the age of 18 to 60 years, presented with signs and symptoms of *Shitada* (Gingivitis) described as per Ayurveda and Modern Science were registered irrespective of their gender, occupation, education and religion.

Exclusion Criteria

Patients below18 years and more than 60 years of age, patients with evidence of malignancy, history of hypersensitivity to the trial drugs, patients taking any other systemic drugs which can alter the result of the study and the patient with periodontal pocket with pus discharge were not included the study.

Investigations

Hematological Examinations (Hb%, TC, DLC, ESR) were carried out before treatment to rule out any systemic disease and after treatment to note any changes in these parameters.

Urine analysis (Physical, Chemical and Microscopic urine examination) and Biochemical –RBS were carried out before treatment to rule out any systemic disease.

Trial drugs

The trial drugs namely *Triphaladi kwatha gandusha*, *Priyangavadi churna pratisarana* and *Triphala churna* were prepared at the pharmacy of GAU, Jamnagar.

Criteria for assessment

Improvement in symptoms obtained in the patients was considered for assessment. A scoring pattern is adopted for assessment of the total effect of the therapy as mentioned below in Tables 1 to 10.

Table 1: *Akasmata rakta srava* (Bleeding from gums)

Akasmata rakta srava	**Score**
Absence of bleeding	0
Slight bleeding on brushing or occasional bleeding	1
Moderate bleeding on brushing or eating hard articles	2
Severe bleeding on brushing or even on chewing food	3
Spontaneous bleeding	4

Table 2: *Vedana* (Pain)

***Vedana* (Pain)**	**Score**
Absence of pain	0
Occasional pain with low intensity-dull ache	1
Frequent pain with a moderate intensity-continuous dull ache	2
Continuous pain with severe intensity which increases during mastication-lancinating pain-radiating type of pain	3

Table 3: *Mukha daurgandhya* (Halitosis)

Mukha daurgandhya	**Score**
Absence of bad odour	0
Slight bad odour which decreases after mouth wash	1
Moderate bad odour rarely decreases after mouth wash	2
Persistent bad odour even after repeated mouth wash	3

Table 4: *Shotha* (Inflammation)

Shotha	**Score**
Absence of inflammation	0
Mild inflammation, a slight change in colour and in the texture of the marginal or papillary gingival unit	1
Moderate inflammation, glazing redness, oedema of the marginal or papillary gingival unit	2
Severe inflammation, marked redness, oedema of the marginal or papillary gingival unit	3

Table 5: *Krishnata* (Discoloration of Gums)

Krishnata	**Score**
Normal (Pinkish Red)	0
Slight discoloration of gums, reddish	1
Moderate discoloration of gums, reddish blue	2
Severe discoloration of gums, bluish red, or blue	3

Table 6: *Dantamamsa prakledata* (Moistness)

Dantamamsa prakledata	Score
Absence of moistness	0
Slight moistness is visible	1
Moderate moistness is visible	2
Severe moistness is visible	3

Table 7: *Paka* (Pus discharge)

Paka	Score
Absence of pus discharge on examination	0
Slight pus discharge on examination	1
Moderate pus discharge on examination	2
Severe pus discharge on examination	3

Table 8: *Dantamamsa mriduta* (Sponginess)

Dantamamsa mriduta	Score
Absence of spongy gums	0
Slight spongy gums	1
Moderate spongy gums	2
Severe spongy gums	3

Table 9: Gingival Index (GI)[7]

Symptom	Score
Absence of inflammation/ normal gingivae	0
Mild inflammation; slight change in colour, slight oedema; no bleeding on probing.	1
Moderate inflammation; redness, oedema, moderate glazing, hypertrophy; bleeding on probing	2
Severe inflammation; marked redness, oedema and hypertrophy; ulceration tendency to spontaneous bleeding.	3

Table 10: Bleeding Index (BI)[7]

Symptom	Score
No bleeding on probing.	0
Bleeding point appear on probing	1
Several isolated bleeding points or a single fine line of blood appears.	2
The interdental triangle fills with blood shortly after probing.	3
Profuse bleeding occurs after probing; blood flows immediately into the marginal sulcus.	4

Statistical Analysis

The therapeutic effects were evaluated in both groups by applying a t-test. The results obtained are considered highly significant for $P<0.001$, significant for $P<0.05$ and insignificant for $P>0.05$.

Observations and Results

Demographic data

The majority of the patients 34.37% belonged to the age group of 18-30 years. 65.62% of the patients were females. 59.37% of patients were having education up to the school level. 81.25% of patients were taking tea as a supplementary diet. 62.5% of the patients were having regular bowel habits. Positive family history was found in 37.5% of patients. All the patients (100%) were using toothbrushes as an oral hygiene method. In 90.62% of the patients, the frequency of cleansing the teeth was only once a day. 93.75% of the patients were practicing improper methods of brushing their teeth. Among the improper method followers, 78.12% of patients had done only horizontal movement of brushing their teeth. In 50% of patients, the frequency of changing their toothbrush was after 6 months. 53.13% of patients were addicted and 46.87% were not addicted to any addiction. Among the addicted patients, 46.87% were addicted to chewing *Pan* (A mixture of areca nut with slaked lime, catechu and other favouring agents). Supari/Betel nut (*Areca catechu*), cigarette and tobacco addiction were observed 9.37% in each. Alcohol and Bidi consuming patients were observed 3.12% in each. 56.25% of patients were found with

Calculous deposition. 43.75% of patients were found with carious teeth and filled teeth. Food impaction was found in 34.37% of patients.

Clinical profile

All the patients (100%) had chief complaints of *Akasmata rakta srava* (bleeding), *Shotha* (inflammation) of gums, *Dantamamsa mriduta* (sponginess), *Krishnata* (discoloration) of gums and *Mukha daurgandhya* (halitosis). 96.87% of patients were having *Dantamamsa prakledata* (Moistness) and 28.12% of patients were having *Vedana* (Pain).

Effect of therapy

Group A

The percentage of relief in *Akasmata rakta srava* (Bleeding) was reduced by 92.85%, *Mukha daurgandhya* (Halitosis) was reduced by 90%, *Shotha* (Inflammation) was reduced by 62.96%, *Krishnata* (Discoloration of gums) was reduced in 86.36% of patients. 82.75% of patients got relief in *Dantamamsa prakledata* (Moistness) and *Dantamamsa mriduta* (Sponginess) was reduced in 65.38% of the patients. All above-mentioned results were statistically highly significant ($p<0.001$) except in *Vedana* (Pain), the relief was 100% of patients and the result was statistically insignificant (>0.05).

Group B

The percentage of relief in *Akasmata rakta srava* (Bleeding) was 51.72%, *Mukha daurgandhya* (Halitosis) was reduced by 92%, *Shotha* (Inflammation) was reduced by 41.4%, *Krishnata* (Discoloration of gums) was reduced in 75% of patients. 55.2% of patients got relief in *Dantamamsa prakledata* (Moistness) and *Dantamamsa mriduta* (Sponginess) was reduced in 61.5% of the patients. All above-mentioned results were statistically highly significant ($p<0.001$) except in *Vedana* (Pain), the relief was 71.42% of patients and the result was statistically significant (<0.05).

Comparative Effect of Therapy

The percentage of relief (Table 11) in *Akasmata rakta srava* (Bleeding) shows better results in Group A (92.85%) compared to Group B (51.72%) and the comparative result was found as statistically highly significant ($p<0.001$). The percentage of relief in *Shotha* (Inflammation) shows better results in Group A (62.96%) compared to Group B (41.4%) and the comparative result was found as statistically significant ($p<0.05$). Group A shows better percentage (82.75%) of relief in *Dantamamsa prakledata* (Moistness) in contrast to Group B (55.2%) and the comparative data was found as statistically significant ($p<0.05$). Group A shows better percentage (86.36%) of relief in *Krishnata* (Discoloration of gums) in comparison to Group B (75%) and the comparative data was found as statistically insignificant ($p>0.05$). Group A shows better percentage (65.38%) of relief in *Dantamamsa mriduta* (Sponginess) in contrast to Group B (61.5%) and the comparative data was found as statistically insignificant ($p>0.05$). Group A showed a 90% of relief percentage in *Mukha daurgandhya* (Halitosis) while it was 92% in Group B. The comparative data is statistically insignificant ($p>0.05$). The percentage of relief in *Vedana* (Pain) shows better results in Group A (100%) compared to Group B (71.42%) and the comparative data was found as statistically insignificant ($p>0.05$).

The percentage of improvement (Table 12) in Gingival Index in Group A was 62.96% and in Group B 41.37% of the patients and the comparative data was found as statistically significant ($p<0.05$).

Group A shows a better percentage of improvement in the Bleeding Index (50%) in comparison to Group B (22.58%) of the patients, which was found as statistically significant ($p<0.05$).

Overall effect of therapy

In Group A, 31.25% of patients obtained complete remission and 62.5% of patients obtained marked improvement. Moderate improvement was 6.25%. There were no patients in the category of mild improvement and unchanged (Figure 1).

In Group B, the percentage of complete remission was 6.25% and marked improvement was 62.5%. Moderate improvement was 25% and mild improvement was 6.25%. There were no patients in the category of unchanged.

Probable Mode of Action of drug combination was presented in Figure 2.

Table 11: Comparative effect of therapy

Complain	Group	N	Mean Diff.	SD [1]±	S.E.±[1]	't '[1]	P[1]	Sign.
Akasmata rrakta srava (Bleeding)	A	16	1.625	0.619	0.155	4.119	<0.001	HS
	B	16	0.938	0.250	0.0625			
Mukha daurgandhya (Halitosis)	A	16	1.688	0.479	0.120	1.426	>0.05	IS
	B	16	1.438	0.512	0.128			
Shotha (Inflammation)	A	16	1.063	0.250	0.0625	2.440	<0.05	S
	B	16	0.750	0.447	0.112			
Krishnata (Discoloration of gums)	A	16	1.188	0.544	0.136	0.338	>0.05	IS
	B	16	1.125	0.500	0.125			
Dantamamsa prakledata (Moistness)	A	16	1.500	0.730	0.183	2.070	<0.05	S
	B	15	1.000	0.632	0.158			
Dantamamsa mriduta (Sponginess)	A	16	1.063	0.929	0.232	0.222	>0.05	IS
	B	16	1.000	0.632	0.158			
Vedana (Pain)	A	03	1.333	0.577	0.333	1.528	>0.05	IS
	B	06	0.833	0.408	0.167			

SD: Standard deviation, SE: Standard error, t: t-test value, P: Significance values

Table 12: The percentage of improvement of the Gingival Index and Bleeding Index

Complaint	Group	N	Mean Diff.	SD ±	S.E.±	't '	P	Sign.
Gingival Index	A	16	1.063	0.250	0.0625	2.440	<0.05	S
	B	16	0.750	0.447	0.112			
Bleeding Index	A	16	1.063	1.063	0.266	2.119	<0.05	S
	B	16	0.438	0.512	0.128			

SD: Standard deviation, SE: Standard error, t: t-test value, P: Significance values

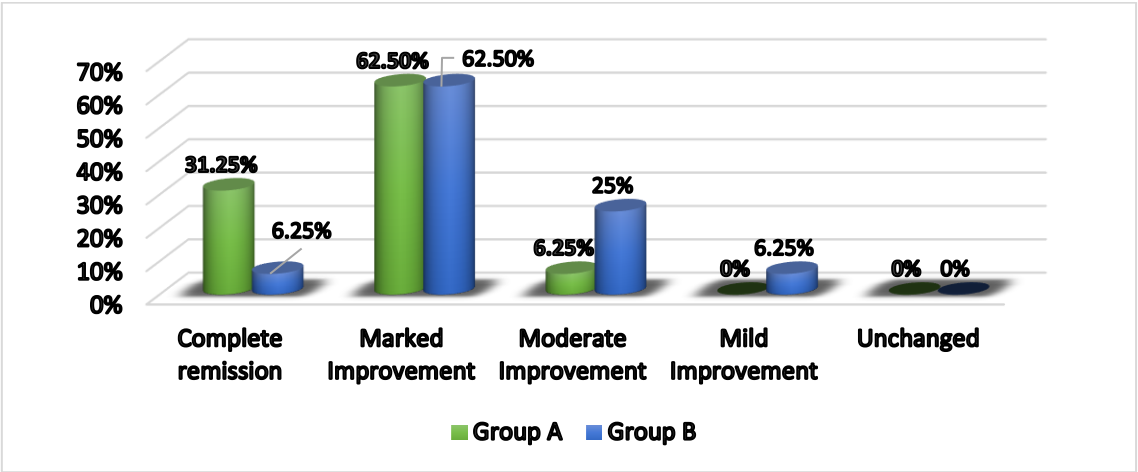

Fig. 1: Overall effect of therapy (n=32)

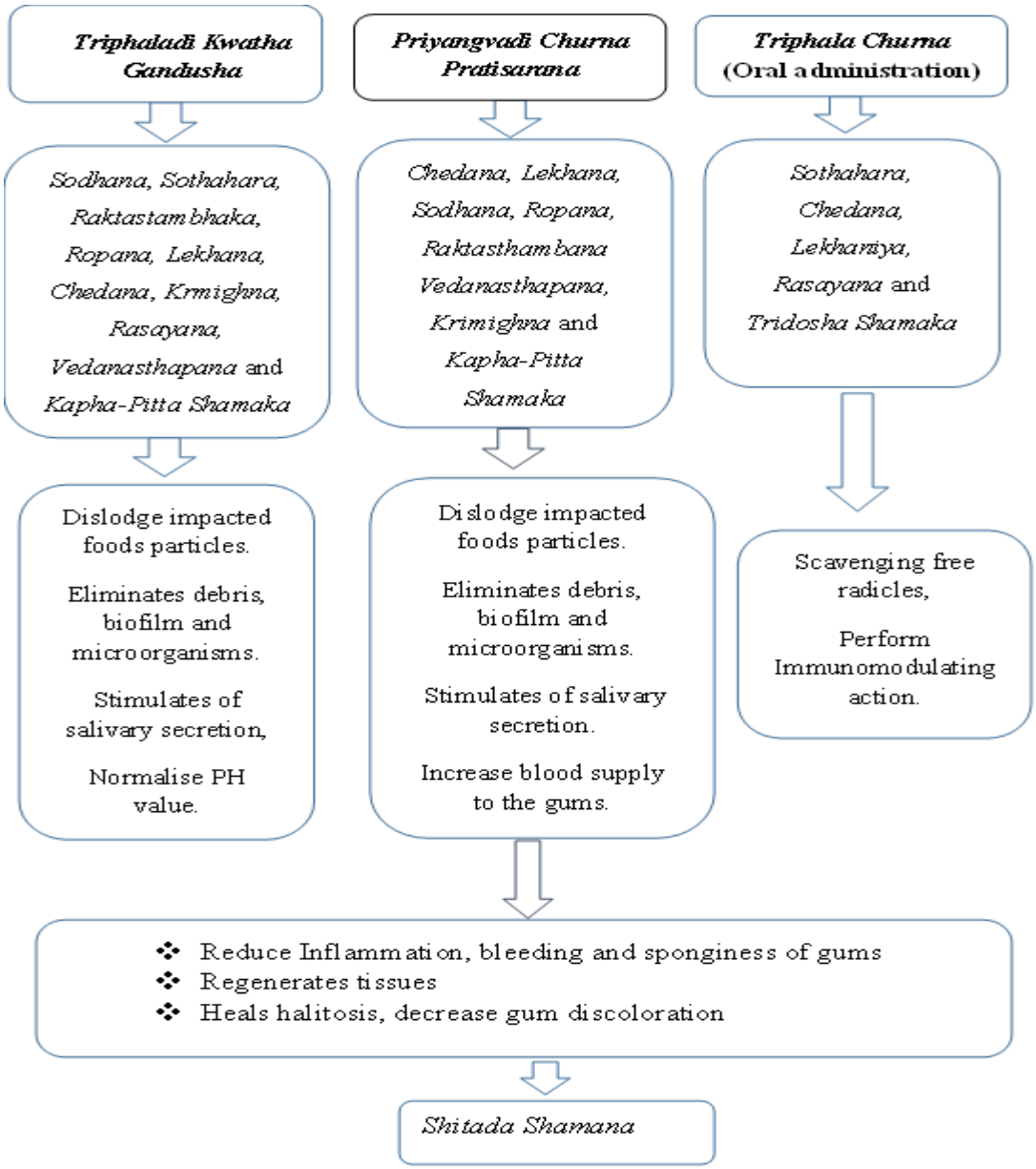

Fig. 2: Probable mode of action of drug combination

Unawareness of the disease condition and improper methods of oral hygiene are seeming to be the higher prevalence in the group. Improper method of brushing the teeth causes inadequate cleaning and results in insufficient removal of plaque leading to Gingivitis. It is said that the toothbrush should be changed every 3 to 4 months, or whenever it appears to be getting worn out. Old toothbrushes are not effective in cleaning the oral cavity properly and fail to eliminate pathogenic microorganisms and plaque sufficiently leading to Gingivitis. Considerable consumption of sweets, oily food, milk and *Takra*etc. leading to vitiation of *Kapha dosha* which can cause *Shitada* (Gingivitis). Sugar is added to tea which is highly favourable for the growth of bacteria in the oral cavity and leads to plaque formation and develops gingivitis[8]. Intake of a diet that is rich in *Madhura rasa*, *Lavana rasa*, *Amala rasa*, *Guru guna* and *Snigdha guna* vitiates *Kapha dosha* and can develop the disease. Addictions increase the risk of developing Gingivitis. Positive family history was found in more than 1/3 of the patients having an influence of genetic predisposition to develop the disease. Calculous-derived endotoxins act as irritants or antigens in both nonspecific acute inflammatory response and immune mechanism of defense which lead to inflammation of gums. Therefore, Calculous plays a causative factor in Gingivitis. Food impaction suggests a positive environment for bacterial colonization and it is a major cause of developing Gingivitis. Discoloration of the gums depends upon the chronicity of Gingivitis. The earliest manifestation of chronic Gingivitis consists of a slight alteration in the colour as Pinkish red and it will progress to red or reddish blue as the hyperemia and inflammatory infiltrate become more intense.

Conclusion

According to this current study, it can be concluded that the administration of *Triphaladi kwatha gandusha*, *Priyangavadi churna pratisarana* with *Triphala churna* orally is more effective than that of administration of *Triphaladi kwatha gandusha*, *Priyangavadi churna pratisarana* alone in the management of *Shitada* (Gingivitis).

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Sudigai (*Agnikarma*) treatment for foot corn: Case report

Sritharan G.[1] and Anpuchelvy S.[2*]

Abstract

Suddigai (*Agnikarma*) means the procedure done with fire. References about *Suddigai* are available in almost all Siddha and Ayurvedic preventive, curative and haemostatic properties were unveiled even centuries back. These same principles are adopted in advanced technologies like cauterization, diathermy, radiation therapy, laser therapy, station device etc. *Suddigai* is the prime para-surgical procedure to treat chronic diseases, which are most difficult to manage because of the antagonistic property of doshas, like the disease caused by *Kapha. Suddigai* is done in neurogenic pain, tendinopathies or in diseases of skin, muscle, vein, ligament, bone or joint where the pain is an exclusive factor. A 64 years old male patient was admitted at Herbal Health Care Centre on 10th April 2017 with complaints of pain and elevated cystic swelling on the right and left sole due to rough and hard footwear. There was no history of direct trauma except that the patient was used to walking barefoot on rough roads. There was a history of excision of elevated layers of corn done repeatedly by the patient himself before visiting us. On the basis of clinical examinations, the patient was diagnosed as a case of corn at the right and left sole. After careful assessment and examination, the patient was treated with *Sudigai*. The Patient completely recovered from foot corns after regular use of *Sudigai* every week. Thus, from the case report it is clear that the Para-surgical procedure of *Agnikarma / Sudigai* helps to treat foot corns. So, *Agnikarma* therapy is more satisfactory in the management of corns.

Keywords: *Suddigai, Agnikarma*, Siddha, Ayurveda, *Kapha.*

Introduction

Calluses and corns are almost similar in origin which reflects as thick, hardened layers of skin that develop when one's own skin develops its own defense mechanism to protect itself against friction and pressure[1]. Corns and callosities are more common in the elderly, not because their skin growth changes but because changes in the skeleton cause redistribution and misdistribution of weight bearing. Callosity is a raised thickened patch of greyish-brown hyperkeratic skin over an area of excessive wear and tear[2]. Thus, they are common on the hands and feet and their site varies with the patient's occupation and skeletal structure. As they exercise a protective function, they are best left alone, but the diagnosis can be confirmed by carefully paring away the top layer of roughened skin to expose the homogeneous, shiny, translucent layers of dead skin beneath[3]. Corn is a similar but smaller lesion that is pushed into the skin. Thus, it forms a palpable nodule with a central yellow–white core of dead cornified epidermal cells. Corns are found on the soles of the feet, the tips of the toes and over the dorsal surface of the interphalangeal joints[4]. The main differential diagnosis is the plantar wart. These two lesions are identified by paring away the top layers of skin with a knife to expose either the corn's core of dead translucent tissue, or the verruca's soft fusiform processes[5]. Prolonged exposure of the skin to sunlight can cause areas of hyperkeratosis of the skin, which cause areas of hyperkeratosis of the skin, which may undergo malignant change. According to Siddha Medicine, corn can be correlated with the disease "*Aanikkoodu*" and Ayurveda the disease '*Kadara*'.

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As per Ayurveda concept, a knotty and painful hard growth raised at the middle or sunk at the sides, which exudes a secretion and resembles an Indian plum and appears on the sole of a person as an outcome of the vitiated condition of the local blood and fat produced by the deranged *Dosha* incidental to the pricking of a thorn or of gravel is called *Kadara*. The growth-like appearance in a nail from the inside due to placing the foot on a rough stone or hurt by thorns etc. is known as *Kadara*. *Kadara* may develop as the vitiation of *Vata* with *Kapha dosha*. *Vata* and *Kapha* have been considered the important factors for the causation of *Shotha* (inflammation) and *Shoola* (Pain)[6]. *Suddigai* (*Agnikarma*) (cauterization) means a procedure done with fire. References about *Suddigai* are available in almost all Siddha, Ayurveda preventive, curative and hemostatic properties were unveiled even centuries back. These same principles are adopted in advanced technologies like cauterization, diathermy, radiation therapy, laser therapy, station device etc. Clinically is the prime para-surgical procedure, to treat chronic diseases, which are most difficult to manage because of the antagonistic property of *Dosha*, like the disease caused by *Vata* and *Kapha*. *Suddigai* is done in neurogenic pain, tendinopathies or in diseases of skin, muscle, vein, ligament, bone or joint where the pain is an exclusive factor. It is also suggested in hyper granulated neurogenic ulcers, sinuses, tumors, hemorrhoids, fistula-in-anus, warts, moles, and trauma to joints and veins[7]. But in internal hemorrhage, multiple wounds and in rupture of internal organs it should not be practiced. Fearful persons, old aged, are also exempted because of their weak mental strength. In persons with dominant Pitta traits of the body and in un-retrieved foreign bodies which is a potent source of infection, the persons contraindicated for *Suddigai pitta*. Based on the part where cauterization is to be done and the ability of a material to retain and transmit heat energy, different instruments are enumerated by the ancient Substances which can retain less heat and can transmit a lesser amount of heat energy is indicated for skin like *Pipper longum*, goat's and cow's tooth, wooden arrow /*Shalala*. Substances that can retain more heat energy and can transmit it to further deeper layers are used for burning the muscles, tendons and ligaments. For e.g. (in a stone carved in the shape of *Eugenia jambolana* Lam.) metals are used for transmitting the heat energy to the level of muscles and honey, jaggery or other viscous materials like oil, fat etc. are used to transmit the heat energy to ligaments[8].

Case report

A 64 years old male patient was admitted to Herbal Health Care Centre on 10th April 2017 with complaints of pain and elevated cystic swelling on the dorsal aspect of the right and left sole due to rough and hard footwear and barefooted over a period of fifteen years. On inquiry, the patient was found to be a case of controlled diabetes for 15 years, however, which become uncontrolled for six to eight months. his blood glucose levels were within normal limits. But in the last two months developed non-healing wounds over the left and right foot. He took treatment for the same from modern medicine surgeons but the prognosis was poor. There was no history of direct trauma except that the patient was used to walking barefoot on rough roads. There was a history of patient excision of elevated layers of corn done repeatedly by the patient himself, before visiting us. On the basis of clinical examination, the patient was diagnosed as a case of corn at the right and left sole. After careful assessment and examination, the patient was treated with *Sudigai* treatment procedure

Materials and Methods

The materials used are *Panchadhatushalak*, Gas Stove, *Triphala* decoction, Gauze pieces, Sponge holding forceps, *Pachaiennai* (Green oil), Aloe vera leaf pulp, *Curcuma churnam*.

Procedure of Suddigai (Agnikarma)

Purva karma (Preoperative procedure)

Inform written consent of the patient was taken after explaining in detail the procedure. Then the patient was allowed to adopt a comfortable position over the operating table as per the site of the lesion. The

site was cleaned with *Triphala* decoction and wiped with a dry sterile cotton gauge to attain asepsis.

Pradhana karma (Para operative procedure)

The surrounding area was draped in a sterile sheet. Then gas was opened and *Suddigai shalaka* was on it and heated till it become red hot. Red hot *Shalaka* was applied on the lesion in *Pratisarana* (a flat type of Cauterization) with the base of *Panchadhatu salaka* followed by *Bindu* (dotted type of cauterization) to burn the "*Annikoodu*" (corn) (Figure 1). Every time *Shalaka* (heated instrument) is applied within the area of corn for 30 seconds. In case needed, the *Shalaka* was reheated and again applied to the lesion after assessment until the whole of the hyperkeratosis tissue was burnt. Soon after *Suddigai karma*, Aloe vera leaf pulp was applied over the site of *Suddigai karma* to reduce the burning sensation. Proper precautions were taken to evade the production of *Asamyak dagdha* (incomplete burn) (Figure 2).

Paschat karma (Postoperative procedure)

After completion of the procedure, the wound was covered with *Curcuma longa* powder and the entire procedure was repeated at the interval of seven days for desirable results. The patient was advised to apply the paste of *Curcuma longa* powder mixed with *Pachaiennai* (green oil) at bedtime up to the normal appearance of the skin.

This procedure was repeated at the interval of 1st week up to 20 weeks (Figures 3,4, and 5).

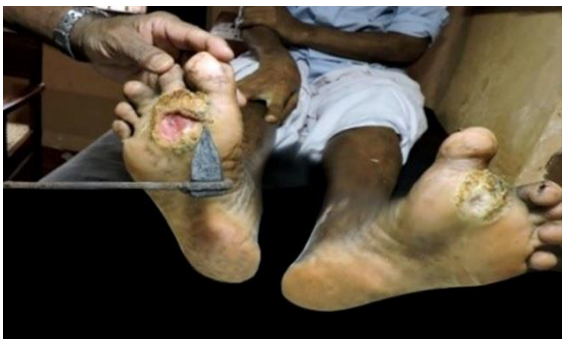

Fig.1: *Suddigai* procedure

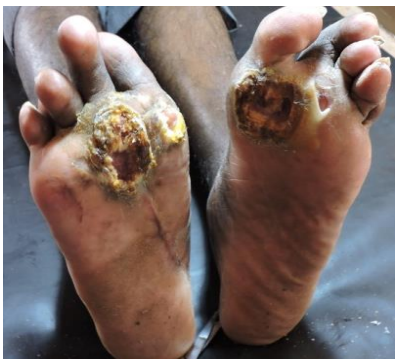

Fig. 2: Before treatment

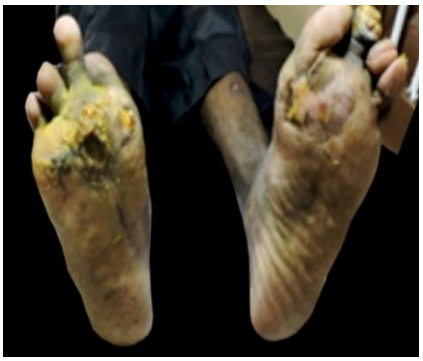

Fig. 3: After 12 weeks of treatment

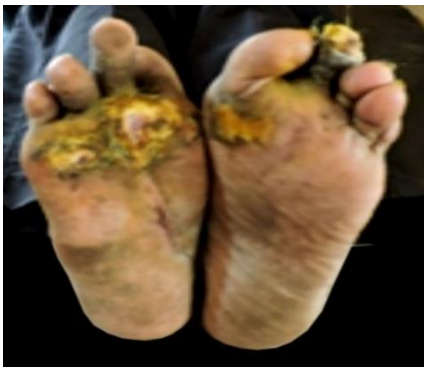

Fig. 4: After 15th weeks of treatment

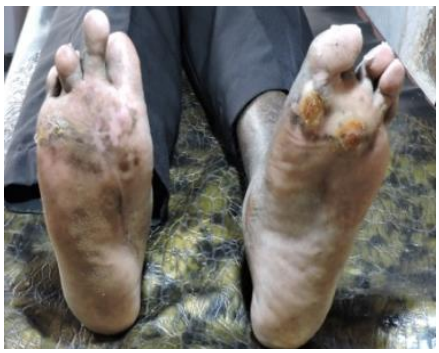

Fig. 5: End of the treatment

Discussion

Suddigai pacifies *Vata* and *Kapha dosha*. When we see the *Nidana* (etiology) of *Kadara, Vata* and *Kapha* are the chief *Dosha* responsible for its manifestation. *Suddigai* helps in decreasing pain by alleviating the vitiated *Vata*. The *Ushna guna* of *Agni* is helpful to reduce the *Sheetha guna* of *Vata. Swedana* is capable of decreasing the symptoms and healing easily, as *Suddigai* is also a *Swedana karma* that heals the lesion completely and there is no recurrence. According to Dr. Ven Hanff the place where heat burns the local tissue metabolism is improved[9]. By the application of heat, it provides additional heat to the tissue which activates the *Dhatwagni* and removes the *Shrotavarodha*. Due to this there is dilatation of blood vessels and improves the circulation to the tissue. Activation of *Dhatwagni* and improved circulation may help in the formation of new healthy tissue. Similarly, Aloe vera is analgesic, anti-inflammatory, antioxidant and wound healing action[10]. It has a quick action on burning sensation as its *Virya* is *Sheeeta* (cold potency) and *Curcuma* powder also has the properties of anti-inflammatory, wound healing, antioxidant and analgesic[11]. Thus, Aloe vera and *Curcuma* help to relieve pain and secondary infection. Acharya Sushruta said that the disease which is not cured by *Bhesaja, Ksahara, Sastra chikitsa,* can be cured by *Agnikarma* and there are no changes of reoccurrence as it itself a sterile procedure. In modern medicine, there is a reoccurrence of corn after the use of medicine locally and it being excised which is painful and time-consuming as well. But after the use of *Agnikarma* (*Suddigai*) there is no change of reoccurrence as well as there is no complication of it. Heat increases local circulation and tissue metabolism, vasodilation, increasing nutrition to the cells and heals the wound completely. The *Agni* is having *Laghu* and *Ushna guna* which helps to relieve the *Shrotorodha* and *Kandu* (itching) by reducing *Kapha*. For *Suddigai* as keeping the heated *Shalaka* over the excised part, because of the heat, it kills the micro-organism also. Along with the unhealthy tissues burn some healthy tissues also by this it avoids recurrence.

Conclusion

The patient is completely recovered from foot corns after regular use of *Suddikai* (*Agnikarma*) every week and Green Oil application every day for a period of 6 months. Thus, from the case report, it is clear that the Para-surgical procedure of *Suddikai* helps to treat foot corns appropriately and in cases with corns to prevent further growth of corns Instead of surgical excision, *Suddikai* therapy is more satisfactory in the management of corns. *Suddika* (*Agnikarma*) is a procedure in which there is no need for local anesthesia, the procedure is not too painful as compared to surgical excision. There is also no chance of bleeding as well. Since there is no chance of recurrence and a cost-effective procedure, *Suddikai* is the best for the "*Aanikkoodu*" or "*Kadara*" (corn)

Conflict of Interest

Not declared

Acknowledgment

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Screening of market cow's ghee samples to detect adulteration

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Abstract

Cow ghee is a nutritious food with a lot of health benefits and popular ingredient in many vegetarian diets and indigenous medical formulations. Due to high demand, the adulteration of cow ghee with more affordable and widely available vegetable oils and animal fat is common in many industries. The detection of adulteration by instrumental techniques is expensive and time-consuming. Therefore, simple, rapid and cost-effective tests are essential for the detection of adulterants in cow ghee. The aim of this study was to compare the physicochemical parameters of pure cow ghee with the market samples using the SLS 313 and detection of adulteration using chromogenic tests and conformation through GC-MS. A pure cow ghee sample (S-01) was prepared from the curd made in the laboratory. Thirteen market samples (S-02 to S-14) were purchased from Northern, Southern, Western and Central provinces and analyzed for physicochemical parameters (moisture and volatile matter content, relative density, refractive index, acid value, iodine value, saponification value and peroxide value) based on SLS 313 standard protocols. As chromogenic tests, Modified Salkowski, and furfural tests were followed. In the Modified Salkowski test, the pure sample observed a red colour, whereas the adulterated samples showed a reddish brown to dark brown colour. The pure sample showed no colour in the furfural test, while the adulterated sample showed a light pink to crimson red. In conclusion, eleven market samples (from S-02 to S-12) were adulterated in different levels with edible oils and GC-MS analysis confirmed the adulterants and the chemical composition variation from the pure cow ghee samples.

Keywords: Adulteration, Chromogenic test, Cow ghee, Standard, Vegetable oil, GC-MS analysis

Introduction

The human food culture is inextricably linked to milk and dairy products. As it contributes good sensory and nutritional qualities as well as economy to milk and other food products, milk fat is a very desirable and expensive substance that has been consumed all over the world since antiquity[1]. Cow ghee is a valuable dairy product which is golden yellow colour[2] and produced from cow milk. It is mostly used for food industries as well as for traditional medicine[3]. Under tropical storage circumstances, it has a lengthy shelf life of approximately one year[4]. It is rich in nutrients and it's beneficial for children as well as old people.

Cow ghee generates good income in dairy industry and trades mostly tend to adulterate[5] the pure cow ghee with cheaper[6] and easily available vegetable oils such as palm oil[7,8], coconut oil[9], mee oil[10], sunflower oil[11], soybean oil[12], sesame oil[13], peanut oil[14] as well as rice bran oil[15] and animal fat[16,17] due to its higher price and the demand[4,18]. Adulteration is the process of reducing the quality or nature of a particular substance by adding an unnatural or subpar substance and removing essential components[19]. Adulteration of cow ghee is difficult

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to identify visually[4] when the adulterants have similar colour and the combination of adulterants with similar chemical composition as pure cow ghee. The detection of adulterants by instrumental techniques (GC-MS, UV spectrophotometer and Electronic sensing nose system) is expensive and time consuming[3]. Simple, rapid and cost-effective tests are essential for the detection of adulterants in cow ghee to facilitate the traders to purchase good quality cow ghee by saving the time and money.

Using the SLS 313 standards for animal and vegetable fats and oils, the objective of the study was to compare the physicochemical parameters (moisture and volatile matter content, relative density, refractive index, acid value, iodine value, saponification value, and peroxide value) between the pure and market samples and develop a colour test for straightforward adulteration detection and conformation through GC-MS. Colour tests are simple, rapid and cost-effective chromogenic tests which can be detect by the layman who is purchasing the ghee to detect the adulteration [20].

Materials and methods

Preparation and sampling of ghee

Pure ghee was prepared from the curd made in the laboratory from cow's milk. Fresh cow's milk (2 L) was purchased from a farm and boiled. After cooling, cow curd (1 table spoon) was added, stirred well and allowed to ferment for overnight. After the curd formed, distilled water (1.5 L) was added and stirred at 300 rpm using the overhead stirrer for 3 hours until the fat separated. The separated fat was collected, kept in the fridge for overnight, melted and bottled (S-01). The samples from S-02 to S-14 were purchased from Northern, Southern, Western, and Central Provinces of Sri Lanka randomly in July 2021 and from January to February 2022.

Preparation of adulterated ghee samples

A 1 mL adulterated ghee sample prepared from pure ghee was mixed with 5 %, 10 %, 15 %, 25 % and 50 % percentages of adulterants (palm oil, mee oil, sesame oil and vegetable ghee) separately and used for chromogenic tests.

Determination of acid value

The pure and market samples (2 g each) were taken in conical flasks and the acid value was determined according to SLS 313-2-6:2009 (hot ethanol method using indicator)[21]. Ethanol was taken into another flask and boiled it until bubbles formed. Phenolphthalein (2 drops) was added and neutralized using potassium hydroxide (KOH) solution (0.1M). Neutralized ethanol (25 mL) was added to sample and shaken well. Phenolphthalein (2 drops) was added and titrated with KOH solution until pink colour formed. Blank test was carried out without the ghee sample.

Determination of saponification value

The saponification value of pure and market samples of cow ghee (2 g each) was determined according to SLS 313-2-1:2014 procedure [21]. Pre-prepared ethanolic KOH (25 mL) was taken in a round bottomed flask, boiling chips added, fixed to the condenser and refluxed for 1-2 hours until clear solution formed. The solution in the flask was titrated with 0.5M hydrochloric acid (HCl) solution after added 2 drops of phenolphthalein indicator. Blank test was done using the same procedure without ghee sample.

Determination of refractive index

Refractive index was determined using refractometer at room temperature according to SLS 313-1-5:2017 procedure [21]. Drop of distilled water was placed on refractometer prism and focused the light. Refractive index of water determined and checked with temperature in standard table. Prism of refractometer was cleaned and placed the drop of ghee sample and determined the refractive index of sample. The same way other samples also analyzed [21].

Determination of iodine value

Samples (0.634-0.793 g each) were taken in different stoppered conical flasks and iodine value was determined according to SLS 313-2-2:2019 procedure [21]. Cyclohexane and glacial acetic acid in 1:1 ratio (20 mL) was added and 25 mL of Wij's

reagent pipetted into the flask. Flask was shaken well and placed in dark for 1 hour. Potassium iodide (100 g/L, 20 mL) and 150 mL of distilled water were added to conical flask. Solution was titrated with 0.1M sodium thiosulphate solution. Near to end point, few drops of saturated starch solution were added and titration was continued until blue colour just disappeared. Blank test was done without test sample.

Determination of relative density

Relative density was measured according to SLS 313-1-2:2009 using specific gravity bottle [21]. Clean and dry specific gravity bottle was weighed, filled with freshly boiled and cooled distilled water. Filled specific gravity bottle was kept in water bath until it reached 40 °C. It was maintained at 40 °C until no further alteration in volume occurs. The bottle was removed from water bath and dried outside and allowed to cool to room temperature (27 °C). Then weight of bottle was measured. The bottle was cleaned and filled with ghee sample and the above procedure followed for other samples.

Determination of moisture and volatile matter content

Moisture and volatile matter content of each sample was determined according to SLS 313-3-5:2016 procedure [21]. Empty vessel was weighed and 5 g of each test sample was added and taken initial weight. Vessels with samples were placed in an oven for 1 hour (103 ± 2 °C). Test samples were cooled to room temperature using desiccator and weighted to nearest 0.001 g. Operations of heating, cooling, weighing were repeated until constant weight.

Determination of peroxide value

Samples (2 g each) were taken into flasks and peroxide values were determined according to SLS 313-3-7:2017 standard procedure [21]. Isooctane (10 mL) and glacial acetic acid (15 mL) were added to sample. Saturated potassium iodide (KI) solution (1 mL) was added and shaken well for 1 minute. Flask was placed in dark for 5 minutes and 75 mL of distilled water added. It was titrated with 0.01M sodium thiosulphate solution. Near to end point, few drops of starch solution was added and titration was continued until solution became colourless. Blank test was done with the same procedure without ghee sample.

Modified Salkowski's test

Ghee sample (1 mL) was taken into test tubes. Petroleum ether (b.p: 40 – 60 °C, 2 mL) was added to the sample and mixed well until clear solution obtained. Concentrated sulfuric acid (5-10 drops) were added, shaken well and the colour change was observed within a minute [22]. This procedure was continued for samples prepared in the laboratory by adulterating with palm oil, mee oil, sesame oil and vegetable ghee separately with pure ghee in 5%, 10%, 15%, 25%, and 50% percentages.

Furfural test

Melted ghee (1 mL) was taken into test tubes and concentrated hydrochloric acid (2 mL) was added to each sample separately and mixed well. Ethanolic furfural solution (2%, 0.1 mL) was added to each sample and kept for 2 minutes [6]. Furfural test procedure was continued for the sample prepared in the laboratory by adulterating pure ghee with sesame oil in 5%, 10%, 15%, 25% and 50% percentages.

Gas chromatography- mass spectrometry analysis of ghee samples (GC-MS)

The ghee sample (0.1 g) was weighted, and 2 mL of iso-octane was added. The sample was well mixed, and 0.1 mL of ethanolic potassium hydroxide (2 mol/L) was added. The sample was shaken vigorously for 1 minute. It was allowed to settle for 2 minutes before adding 2 mL of saturated sodium chloride solution. After shaking the sample, the top layer (iso-octane) was separated. Anhydrous sodium sulphate (1 g) was added to the separated top layer and filtered. The supernatant was transferred to a glass vial and used for GC-MS analysis. The samples were qualitatively analyzed using a Gas Chromatograph (Thermo Scientific TRACE 1300) together with an MS (ISQ-QD, Single Quadrupole) connected with an auto-injector AI 1310 (Thermo

Scientific) and a length of 30 m, diameter of 0.25 mm, and 0.25 µm film Thermo Scientific fused silica capillary column (DB-WAX UI) with a flow of 1.0 mL/minute. The following temperature programming was used: starting temperature of 60 °C, ramping up to 225 °C at a rate of 5 °C/minute. The ion source temperature was 250 °C, and 240 °C was the injector temperature. A pre-prepared sample was injected at 5.0 mL/minute split flow and 64.20 kPa column pressure. Ion capture detector with impact energies of 70 eV was used for the MS. The constituents were identified by comparing the mass spectra to spectra from the equipment database (NIST 11).

Results

Physicochemical parameters

The hysicochemical parameters are evaluated to deterine the quality of ghee. They are moisture and volatile matter, relative density, refractive index, acid value, iodine value, saponification value and peroxide value (Table 1).

Modified Salkowski's test as a chromogenic test

Table 2 shows the Modified Salkowski's test results of adulterated ghee with palm oil, mee oil, sesame oil, vegetable ghee (VG)

Modified Salkowski's test results of pure ghee adulterated with palm oil (Figure 1), Modified Salkowski's test results of pure ghee adulterated with mee oil (Figure 2), Modified Salkowski's test results of pure ghee adulterated with sesame oil (Figure 3) and Figure 4 shows the Modified Salkowski's test results of pure ghee adulterated with vegetable ghee (VG).

Furfural test as a chromogenic test

Furfural test results of pure ghee adulterated with sesame oil shows in Table 3 and Figure 5.

Results of GC-MS analysis of S-01, S-10, S-13, S-14, Buffalo ghee, palm oil, sesame oil, and vegetable ghee is shown in Table 4.

Figure 6 shows the GC-MS graph of pure cow ghee (S-01).

Table 1: Physicochemical parameter values of pure ghee and market samples

Sample	Moisture & Volatile matter %	Relative Density	Refractive index at 28 ˚C	Acid value (mg KOH/g)	Iodine value % by mass	Saponification value	Peroxide value meq. (O_2)/kg of ghee
S-01	0.21±0.02	0.073±0.001	1.4581±0.0002	0.5±0.07	35±0.10	230±0.18	3±0.12
S-02	0.44±0.02	0.073±0.001	1.4559±0.0001	5.7±0.18	30±0.26	243±0.20	3±0.06
S-03	0.23±0.01	0.072±0.002	1.4551±0.0001	4.5±0.20	31±0.19	236±0.09	68±0.21
S-04	1.88±0.02	0.081±0.001	1.4581±0.0002	27.8±0.23	20±0.27	242±0.20	172±1.00
S-05	0.28±0.02	0.073±0.001	1.4581±0.0002	8.4±0.07	29±0.21	237±0.16	40±0.15
S-06	0.12±0.01	0.074±0.002	1.4612±0.0001	0.6±0.11	ND	202±0.20	ND
S-07	0.35±0.01	0.073±0.001	1.4600±0.0002	6.7±0.07	30±0.21	245±0.19	10±0.25
S-08	0.30±0.02	0.072±0.002	1.4574±0.0001	2.2±0.22	31±0.13	208±0.12	12±0.12
S-09	0.15±0.02	0.080±0.002	1.4551±0.0001	0.8±0.09	32±0.18	216±0.12	12±0.25
S-10	0.16±0.02	0.078±0.002	1.4576±0.0002	1.7±0.11	33±0.12	238±0.24	9±0.58
S-11	0.35±0.01	0.074±0.002	1.4574±0.0002	3.9±0.13	28±0.20	240±0.17	22±0.76
S-12	0.31±0.01	0.069±0.001	1.4587±0.0002	1.7±0.12	31±0.18	239±0.20	10±0.81
S-13	0.21±0.01	0.073±0.001	1.4580±0.0001	0.8±0.07	36±0.17	220±0.19	3±0.17
S-14	0.23±0.01	0.073±0.002	1.4578±0.0002	0.7±0.14	31±0.23	228±0.16	3±0.00

ND- Not determined

Table 2: Modified Salkowski's test results of adulterated ghee with palm oil, mee oil, sesame oil, vegetable ghee (VG)

1 mL of Sample		Observation			
Pure Ghee (%)	**Adulterant (%)**	**Palm oil**	**Mee oil**	**Sesame oil**	**Vegetable ghee**
100	-	Red colour	Red colour	Red colour	Red colour
95	5	Reddish brown colour	Reddish brown colour	Reddish brown colour	Reddish brown colour
90	10	Reddish brown colour	Reddish brown colour	Reddish brown colour	Reddish brown colour
85	15	Brown colour	Brown colour	Brown colour	Brown colour
75	25	Dark brown colour	Dark brown colour	Dark brown colour	Dark brown colour
50	50	Dark brown colour	Dark brown colour	Dark brown colour	Dark brown colour

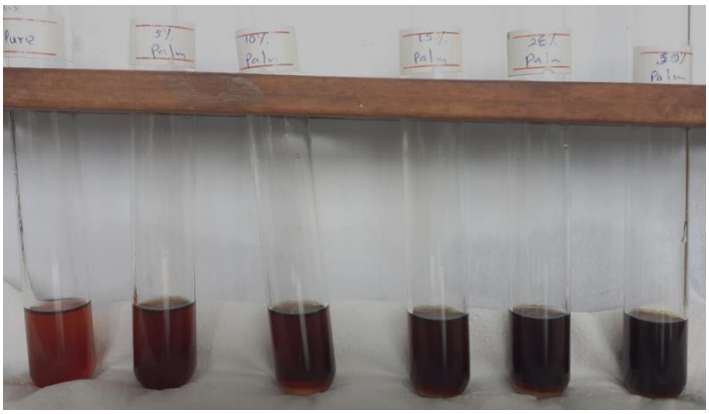

Fig. 1: Modified Salkowski's test results of pure ghee adulterated with palm oil

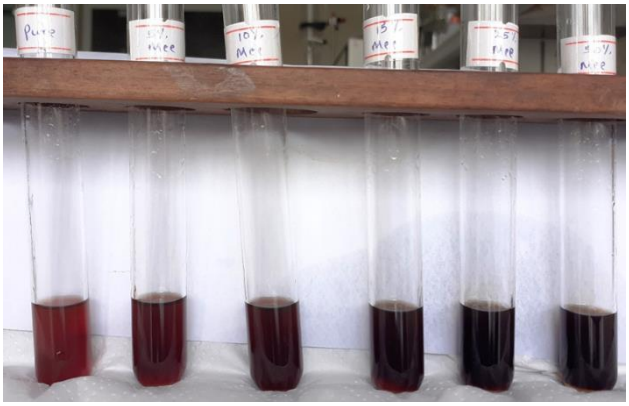

Fig. 2: Modified Salkowski's test results of pure ghee adulterated with mee oil

Fig. 3: Modified Salkowski's test results of pure ghee adulterated with sesame oil

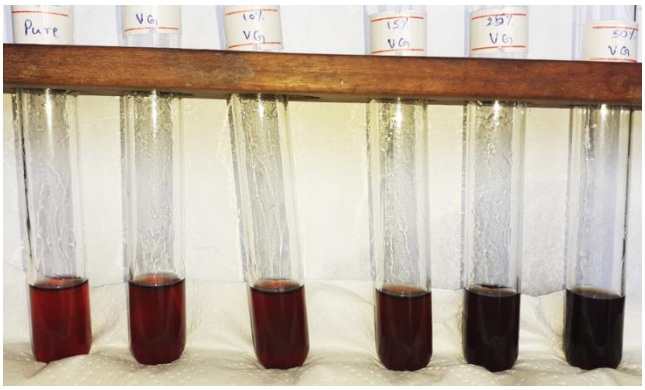

Fig. 4: Modified Salkowski's test results of pure ghee adulterated with vegetable ghee (VG)

Table 3: Furfural test results of pure ghee adulterated with sesame oil

Sample	Observation
Pure Ghee	Colourless
Pure Ghee : Sesame oil (95:5)	Light pink colour
Pure Ghee : Sesame oil (90:10)	Light crimson red colour
Pure Ghee : Sesame oil (85:15)	Light crimson red colour
Pure Ghee : Sesame oil (75:25)	Crimson red colour
Pure Ghee : Sesame oil (50:50)	Crimson red colour

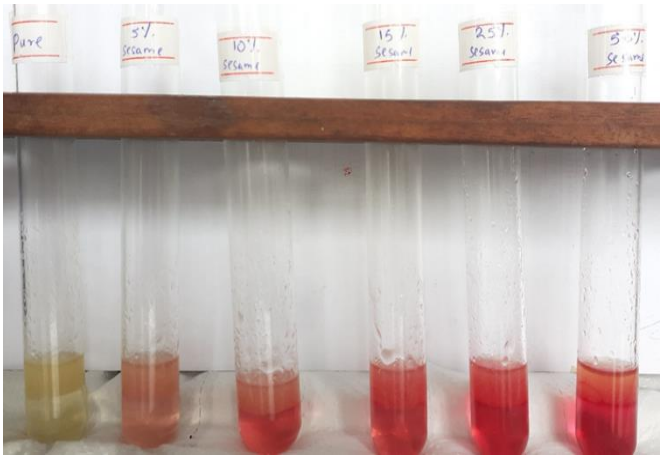

Fig. 5: Furfural test results of pure ghee sample adulterated with sesame oil

Table 4: Results of GC-MS analysis of S-01, S-10, S-13, S-14, Buffalo ghee, palm oil, sesame oil, and vegetable ghee.

Compounds	Relative abundance (%)							
	S-01	S-10	S-13	S-14	Buffalo ghee	Palm oil	Sesame oil	Vegetable ghee
Caproic acid (C6:0)	1.31	1.75	1.36	0.02	0.01	-	-	0.24
Caprylic acid (C8:0)	0.88	0.99	0.94	0.05	0.17	-	0.04	2.83
Capric acid (C10:0)	1.61	2.02	2.10	0.06	0.14	-	0.02	1.99
Undecylenic acid (C11:1n1)	0.11	0.17	0.18	-	-	-	-	-
Lauric acid (C12:0)	2.63	3.91	3.18	0.42	1.45	-	0.25	17.44
Tridecanoic acid (C13:0)	-	0.03	0.05	-	-	-	-	-
Myristic acid (C14:0)	7.66	11.36	10.23	1.26	1.51	0.01	0.11	7.89
Myristoleic acid (C14:1)	0.37	0.82	0.74	0.01	-	-	-	-
Pentadecanoic acid (C15:0)	0.52	1.07	1.13	0.06	0.03	-	-	-
Octadehydroarachidonic acid	-	-	-	-	0.02	-	-	-
Palmitic acid (C16:0)	26.95	30.70	29.08	42.69	41.77	9.85	9.55	29.48
Palmitoleic acid (C16:1)	0.91	1.41	1.44	0.18	0.13	0.13	0.11	0.06
Margaric acid (C17:0)	0.37	0.48	0.77	0.10	0.06	0.02	0.02	0.03
Stearic acid (C18:0)	17.78	10.25	14.27	4.84	4.96	5.93	5.46	3.63
Oleic acid (C18:1)	27.72	18.84	21.92	36.67	37.74	38.51	36.34	28.37
Vaccenic acid (C18:1)	3.50	3.31	0.59	-	1.03	0.01	-	0.64
Linoleic acid (***C18:2***)	2.82	0.81	2.07	11.06	8.63	42.83	45.37	4.74
Nonadecanoic acid (C19:0)	0.04	-	0.11	-	-	-	-	-
Linolenic acid (C18:3)	0.13	0.21	0.36	0.10	0.02	0.30	0.34	0.04
Arachidic acid (C20:0)	0.17	-	0.23	0.35	0.30	0.66	0.54	0.17
Gondoic acid (20:1)	-	0.98	0.05	-	-	-	-	-
8,11,14-Eicosatrienoic acid (C20:3)	-	-	0.04	-	-	-	-	-
Heneicosanoic acid (C21:0)	-	-	0.03	-	-	-	-	-
Arachidonic acid (C20:4)	0.05	-	0.08	-	-	-	-	-
Stearolic acid (C18:1)	-	3.09	-	-	-	-	-	-
Behenic acid (C22:0)	-	-	0.06	0.04	-	0.08	0.09	-

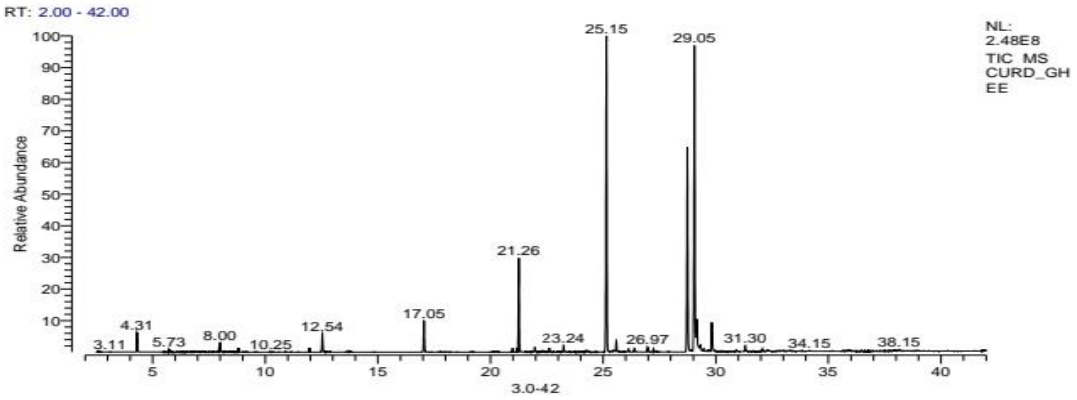

Fig. 6: GC-MS graph of pure cow ghee (S-01)

Discussion

Pure ghee

One of the most essential dairy products is ghee, which is clarified butter[17] made over heat. By taking off the water, protein, and other minor components, it can be made from cream or butter[23]. In the Middle East and South Asian nations, ghee is widely available and used often[1]. The quality of ghee is influenced by the types of milk, cream, *Dahi* (curd), and butter used in its processing, the temperature at which it is clarified, the storage environment, and the animal feed used[23,24]. The yield of cow ghee depends on the fat content of milk which was used to manufacture the ghee. The fat content of cow milk varies from 3 to 4% [25]. The yield of laboratory made ghee (S-01) was 3%. Cow's milk (2 L) gave 60 mL of pure cow ghee. In contrast to cow ghee, which is golden yellow in colour[2], buffalo milk contains no carotenoids, hence the ghee made from it is white [23].

Physicochemical parameters

The physicochemical parameters were determined to the pure ghee and market samples to see how the market sample values are deviating from the pure cow ghee due to adulteration. In specification for ghee (butter oil) standard, SLS 340:1975, maximum moisture content of ghee should be 0.25%. The moisture content of S-01, S-03, S-06, S-09, S-10, S-13 and S-14 were below 0.25% according to the standard. When the molecular weight and unsaturation of oil increase, density also increases. The high relative densities may be a sign of high molecular weight and unsaturation[26]. Sample S-04, S-09, and S-12 were highly deviating from the S-01 (Table 1).The refractive index is a crucial optical statistic for analyzing how light travels through different types of media[27]. According to SLS 313-1-5:2017, refractive index ranges from 1.3000 to 1.7000. In this study, the refractive index of S-01 was 1.4581. Samples S-06 (1.4612) and S-07 (1.4600) were highly deviating from the value for pure ghee. The free fatty acids in oil are quantified by their acid value. The level of free fatty acids [28] increase is directly proportional to the acid value, which results in lower oil quality[29]. Acid value for ghee ranged from 0.0 to 1.0[4]. The acid value of samples S-02, S-03, S-04, S-05, S-07, S-08, S-10, S-11 and S-12 were highly deviating from the value for pure ghee. The degree of unsaturation is measured by the Iodine value, which is frequently used to describe fats and oils[30]. As an oil is oxidized, a decrease in the iodine value is consistent with a decrease in double bonds[31]. Iodine value was determined to identify the unsaturation in the ghee [32]. According to SLS 313-2-2:2019, the iodine value should be between 3.0 and 200.0 for edible oil and fat. The S-01 had 35 and the S-06 didn't show any

colour change during titration, which may be due to the added synthetic orange dye. The molecular weights of the triglycerides in oil are shown by the saponification value. Since the saponification value is inversely related to the average molecular weight or chain length of the fatty acids, higher saponification value denotes a higher fraction of lower fatty acids[29]. In specification for ghee (butter oil) standard, SLS 340:1975, the saponification value of pure ghee should be between 218 and 234. The results obtained for pure sample was 230 (S-01) and two market samples showed results within range, 220 (S-13) and 228 (S-14). Other market samples (S-02 to S-12) values were out of this range indicated that they were adulterated. Peroxides are one of the byproducts of the oxidation of the double bonds in unsaturated lipids. The peroxide value, which is a measure of oxidation level, is high because there is more oxidation. In this study, S-01, S-02, S-13 and S-14 showed peroxide value 3 meq/kg and S-07, S-10, and S-12 showed closer to 10 meq/kg. Due to the added synthetic dye, the sample S-06 did not show any colour change during titration. Among these samples, the physicochemical parameter values of S-04 sample were highly deviated due to the long-term storage (>1 year). Sample S-13 and S-14 showed values closer to S-01 indicated that they are pure. Samples S-02 to S-12 had deviated results when compared to S-01 because they were adulterated with adulterants.

Chromogenic tests

Chromogenic tests show colour change when the reagents are added to the test samples. Modified Salkowski's test gives positive results for cholesterol and plant steroids (phytosterols). The natural pure ghee contains cholesterol but no phytosterols, but the vegetable oil contains phytosterols[12]. Pure ghee gave red colour due to the presence of cholesterol[33]. Phytosterols show a brown ring in Salkowski's test[34]. When pure ghee was adulterated with vegetable oil, the presence of phytosterols gave it a reddish brown colour, which turned dark brown when the amount of phytosterols increased. Sample S-01, S-13 and S-14 gave red colour only but other samples gave reddish brown to dark brown colour. The colour different from red colour indicated that the samples S-02 to S-12 were adulterated with vegetable oils. Sample S-08, S-09 and S-10 showed reddish brown colour indicated that they were adulterated with 5-10 % of vegetable oils.

Furfural test was mainly done for the ghee adulterated with sesame oil (*Vanaspathi* ghee). Sesame oil contains sesamolin which breaks into sesamol and oxonium ion when concentrated hydrochloric acid added[6]. This sesamol reacted with furfural and produced crimson red colour[35,36]. If pure ghee adulterated with sesame oil, it gives crimson red colour. Furfural test was done for 1 mL of pure ghee sample adulterated with sesame oil in different percentages. The colour intensity increased when the sesame oil percentage increased. Sample S-03 and S-05 gave light pink colour and S-07 had light crimson red colour. Therefore, those samples also adulterated with sesame oil. The rest of the samples (S-01, S-02, S-04, S-06, S-08, S-09, S-10, S-11, S-12, S-13, and S-14) were not showed crimson colour, indicating that those samples were not adulterated with sesame oil.

Compounds detected in GC-MS analysis of ghee samples

The analytical technique known as gas chromatography-mass spectrometry (GC-MS) combines the advantages of gas chromatography and mass spectrometry to identify various compounds in a test sample[37]. Pure cow ghee sample (S-01) contains caproic, caprylic, capric, Undecylenic, lauric, myristic, myristoleic, pentadecanoic, palmitic, palmitoleic, margaric, stearic, oleic, vaccenic, linoleic, nonadecanoic, linolenic, arachidic, and arachidonic acid as fatty acids. Oleic acid is the fatty acid with the highest proportion. The other two greatest percentages are palmitic and stearic acid respectively. According to the S-01, market samples S-13 and S-14 gave closer results to it, which indicates those were pure.

In market sample (S-10), palmitic acid shows highest percentage within these fatty acids. The

oleic acid percentage of the market sample (S-10) is lower than that of pure cow ghee (S-01) when compared to pure ghee. Gondoic acid and stearolic acid are present in the S-10 sample. Gondoic acid was detected in S-10 in considerable percentages (0.98%), but only in small amounts (0.05%) in S-13, suggesting that the latter sample may have been contaminated with plant oil. It is commonly found in plant oils such as those from the Fabaceae family [38,39]. Stearolic acid was found in high concentrations (3.09%) in market sample (S-10) and is commonly found in plants. That indicate the S-10 market sample was adulterated with plant oil [40].

The fatty acid composition of buffalo ghee was shown to be closer to that of pure cow ghee, but in different percentages. Undecylenic, Tridecanoic, myristoleic, nonadecylic, and arachidonic acid were not present in buffalo ghee as they were in pure ghee (S-01). Buffalo ghee contains high amounts of palmitic (41.77%) and oleic acid (37.74%).

Palm oil contains myristic, palmitic, palmitoleic, margaric, stearic, oleic, vaccenic, linoleic, linolenic, arachidic, and behenic acid only according to results. Linoleic acid showed the highest percentage (42.83%) among those fatty acids. Comparing sesame oil to pure cow ghee, linoleic acid was more prevalent in sesame oil. The following acids can be found in vegetable ghee: caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, vaccenic acid, linoleic acid, linolenic acid, and arachidic acid. Compared to pure cow ghee, lauric and linoleic acids were present in significant concentrations.

Conclusion

According to the results obtained in the physicochemical parameter tests for the market samples deviating from the pure ghee samples indicated that they appear to have adulterated with different degree of adulteration and chromogenic tests confirmed it. The GC-MS analysis is helpful to identify the adulterants and the variation of chemical composition among adulterated samples. The chromogenic test developed in this research with different percentage of several adulterants will be helpful to do the test on the spot when the customer purchase the ghee sample.

Acknowledgement

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In- vitro study to evaluate the antibacterial activity of *Seethodaka, Visarpahara* and *Neelyadi* oil against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Ayurvedic and traditional oil plays a major role in internal and external treatment for numerous diseases as well as wellness in Ayurveda. Ayurvedic studies have gifted thousands of oil recipes with dynamic applications. *Seethodaka, Neelyadi*, and *Visarpahara* are some of the commonly available traditional oils which show wondering effects in treating skin diseases such as ulcers, wounds, rashes and boils. *Staphylococcus aureus* and *Escherichia coli* are the widely detected bacteria in skin conditions. This study conveys the determination of antimicrobial effects of *Seethodaka*, *Visarpahara*, and *Neelyadi* oils against *Staphylococcus aureus* and *Escherichia coli* using standard antibiotic sensitivity tests. Microbial assays were conducted using Mueller Hinton Agar medium**.** Five wells were made in each plate, and the bottom was sealed using molten agar. Tetracycline or Azithromycin and DMSO were used as positive and negative controls, respectively. The zone of inhibition was measured after incubation at 37°C for 24 hours. Each assay was done in triplicate and the average zone of inhibitions was calculated. The results exhibit an inhibition diameter of 12 mm for *Seethodaka* oil against *Staphylococcus aureus* while 16 mm for *Visarpahara* oil and 13 mm for *Neelyadi* oil against the same bacteria. The positive controller, Tetracycline, shows a 40mm inhibition zone diameter against *Staphylococcus aureus*. According to the results, all three oils have a considerable antimicrobial effect against *Staphylococcus aureus*. Also, 8mm of inhibition zone diameter shows for *Seethodaka* oil for *Escherichia coli*, and there is an incredible antimicrobial effect shown by *Visarpahara* oil against *Escherichia coli* with an inhibition zone diameter of 24 mm. *Neelyadi* oil also has a considerable inhibition zone diameter (12mm) against *Escherichia coli*. The inhibition zone diameter of the positive controller against *Escherichia coli* is 30mm. Therefore, it can be concluded that all three tested traditional oils have considerable antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Staphylococcus aureus, Escherichia coli, Seethodaka, Neelyadi, Visarpahara*

Introduction

Ayurveda is a natural curative medical system with philosophical surroundings based on preventing and curing disease unbroken for thousands of years[1]. The knowledge of Ayurveda passes from teacher to student. This huge science of medicine originated in India; however, unfortunately, due to British attacks, it became discouraged. There are eight branches of Ayurveda. *Kayachikitsa* (medicine), *Shalakya* (dealing with diseases of the supraclavicular region), *Shalya* (dealing with extraction of foreign bodies), *Vishagaravairodika prashamana* (dealing with alleviation of poisons), *Butha vidya* (dealing with spirits), *Kaumarabhritya* (paediatrics), *Rasayana* (promotive methods), and *Vajikarana* (aphrodisiac). Five elemental theories (earth, water, fire, air, and space) and the *Thridoshaja* concept (*Vata, Pitta*, *Kapha*) can be considered as the basis of Ayurveda[1].

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Ayurveda has given guidelines to humans to achieve successful life with good health. It not just deals with the physical body but importantly bonding with mental health. There are four goals of Ayurveda known as *Purushartha* which need to fulfill happy life. *Dharma, Artha, Kama* and *Moksha* are them[1]. Those concepts had broadly discussed in ancient Ayurvedic tests such as Charaka Samhitha, Susrutha Samhitha, and Ashtanga Hrdaya Samhitha, which are known as a greater triad (*Vrhatrai*) as well as less triad (*Laghutrai*). Curing diseases is one of the aims of Ayurveda. Different types of methods of treating various diseases have been described by different Acharyas. For those treatments, ancient Acharyas prepared several medicinal preparations processes known as *Bhaishajya kalpana. Bhaishajya* means a drug, and *Kalpana* means processing. With the ability of formulation, a poisonous drug can be transferred to a non-poisonous and valuable drug. Non-potentate drugs can become potent ones. Therefore, Ayurveda gave great emphasis to drug preparations under *Rasa shasthra* and *Bhaishajya kalpana*. Bhaishajya Rathnavali is a golden classic consisting of hundreds of Ayurvedic preparations written by Kavi Raj Govinda das[2]. Oral medications (*Vati, Kalka, Churna, Kwatha* and *Avaleha*), ointments, oil preparations, eye drops, medicinal cigars, nasal medications, and suppositories are commonly used Ayurveda preparations for several diseases[2].

Among all the preparations, medicated oil is more popular in therapeutic uses[3]. Base oil, decoction, and *Kalka dravya* need for preparing the medicated oil. Prior to the process, crude *Thaila* should follow a special procedure known as *Thaila murchana*[3]. This process enhances the potency of oil and removes bad odour and *Ama dosha* of the oil[3]. Medicated oil is literally used externally as well as internally, for a large number of diseases. Hemiplegia, frozen shoulder, diseases of the oral cavity, diseases of the ear, dental problems, facial paralysis, greying hair, baldness, skin diseases, sciatica, ulcers, eczema, and psoriasis are a few of them. Ayurvedic and traditional oil has a major role in the number of treatments of Ayurveda. It has outstanding pharmacological actions which reach high results in a wide range of diseases. There are thousands of oil recipes can be found in ancient Ayurvedic tests as well as traditional tests. Aushada Samgrahaya, Purana Rahas Thel Beth Potha, Yoga Ghnadeepani, and some ola leaves reveal valuable recipes oils in traditional Sri Lankan medicine while Bhaishajya Rathnawali is rich with valuable Ayurvedic recipes. Various herbal oil use in ayurveda for *Abhyanga* (massage), *Nasya* (nasal drops), *Shiro dhara*, *Sarvangadhara*, *Kavala, Ghandusha* and also used in the application for skin diseases. *Pinda* oil, *Seethodaka* oil, *Jaathyadi* oil, *Neelyadi* oil, and *Sarvavishadi* oil are commonly used for skin diseases in Sri Lanka.

In the present context, human beings are highly susceptible to a variety of skin diseases. It may be due to infection, allergies, autoimmune diseases, and ulcers caused by an external force and ulcers caused by some diseases such as diabetes. Shingles, urticaria, psoriasis, eczema, blisters, rashes, acne, and diabetic ulcers are some of the common skin lesions. Skin infection is common among all skin diseases, which range from mild to life-threatening. According to dermatologists, there are four types of skin infections: bacterial, viral, fungal, and parasitic. Cellulitis, impetigo, boils, and leprosy area few of the most common bacterial infections, while shingles, chicken pox, and measles are common viral infections. Yeast infection, athlete's foot, ringworm, and oral thrush can be considered as common fungal infections. Bed bugs, scabies and cutaneous larva migrants are considered as common parasitic infections[4].

Microbes are highly abundant in common environmental niches and perform various roles to balance the environment. In humans and animals, they provide vast protection against foreign bodies, help to build up immunity, produce some vitamins, and facilitate the digestive process. But researchers have identified that a number of microbes, and their genes badly affect humans and produce diseases[5]. Skin is the first barrier against foreign invaders and also it is the habitat for a diverse population of microbes. *Staphylococcus aureus* (*S. aureus*),

Streptococcus pyogenes (*S. pyogenes*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Candida albicans* (*C. albicans*), and *Escherichia coli* (*E. coli*) can be considered as common pathogenic microbes that grow over skin lesions[5]. *S. aureus* is a gram-positive facultative anaerobe bacterium. Sometimes it may be a skin commensal in approximately 20% of the population[5]. *S. aureus* produces multiple enzymes as well as toxins to attack the host immune system and establish infection. Pseudomonas aeruginosa is a gram-negative opportunistic bacterium that commonly infects patients with cystic fibrosis, burn wounds, COPD, cancer, and immunodeficiency[6]. *P. aeruginosa* infection is difficult to treat due to natural resistance of it. It shows resistance to commonly used antibiotics such as aminoglycosides, carbapenems, and cephalosporins[6]. *E. coli* is a gram-negative bacteria and an important member of the normal intestinal microflora of mammals. According to several research findings, Ayurvedic and traditional medicinal preparations are supposed to have incredible antimicrobial effects. This study is oriented towards the evaluation and comparison of the antibacterial activity of *Seethodaka* oil, *Visarpahara* oil, and *Neelyadi* oil.

Seethodaka oil is a traditional oil which commonly practices in traditional medicine for skin diseases (rashes, ulcers, itching). The main ingredients of *Seethodaka* oil are the *Kaha* (*Acorus calamus*), *Hatavariya* (*Asperagus racemosus*), and *Kohomba* (*Azadirachta indica*) with coconut oil. A paste added to the main ingredient is known as *Kalka* which is made with *Kottamalli* (*Coriandrum sativum*), *Sadikka* (*Myristica fragrans*), *Suduru* (*Cuminum cyminum*), *Kaluduru* (*Nigella sativa*), *Tippili* (*Piper longum*), and *Karabu* (*Syzygium aromaticum*)[7]. *Acorus calamus* is one of the major ingredients in *Seethodaka* oil. In Ayurveda, *Acorus* is considered a plant with an outstanding germ-killing effect. Acorus wear as a bracelet in Asian countries to protect from minute germs[8]. *Asparagus racemosus* is another major ingredient found in the aforementioned oil. According to Ayurveda, the common therapeutic indications of *Asparagus* are skin diseases, skin rashes, epilepsy, urinary disorders, gynaecological problems, improper lactation, and joint disorders[8]. *Azadirachta indica*, another major compound, has common therapeutic indications for skin diseases, itching, burning sensation, anorexia, fever, and blood disorders. *Visarpahara* oil is also a herbo-mineral traditional oil that is commonly used in a number of skin diseases. *Manoshila* (Arsenic sulphide), *Gandaka* (Sulphur), *Palmanikkam* (Copper sulphate), *Sivanguru* (Fe_2O_3), *Sadikka* and *Vasavasi* (*Myristica fragnance*), *Suduru* (*Cuminum cyminum*), *Masakka (Hyssopus officinalis)*, *Ulaarisi (Buceras foenum)*, *Kaluduru* (*Nigella sativa*), *Tippili* (*Pipper longum*), *Chopachini* (*Smilax glabra*), *Karabu* (*Zyzygium aromaticum*), *Katukarosana* (*Picrorhiza kurroa*), *Uluhal* (*Trigonella foenum)*, *Valangasal* (*Embelia ribs*), *Asamodagam* (*pimpinella anisum)*, *Heen aratta* (*Alpenia calcarata*) are the ingredients of *Visarpahara* oil. Almost all the ingredients show excellent effects on skin diseases individually and are commonly used in traditional medicine. Out of all ingredients *Gandaka* (Sulphur), *Palmanikkam* (Copper sulfate), and *Cuminum cyminum* have a great curative effect on skin diseases[9,10]. *Neelyadi* oil is a precious traditional oil that is highly applicable for a wide range of medical conditions such as skin rashes, ulcers, dermatitis, headache, and scalp diseases as well as it is commonly used in traditional *Kadumbindum wedakama* (medicines for fractures). Major ingredients of *Neelyadi* oil are, *Madatiya* leaves and bark (*Adenanthera pavonina*), *Ankenda* leaves and bark (*Acronychia pedunculata*), *Vel keppetiya* leaves and bark (*Croton coudatus*), *Diyahabarala* (*Monchoria vaginalis*), Coconut milk, Coconut oil, *Sudulunu* (*Allium sativum*), *Kaluduru* (*Nigella sativa*) and *Sududuru* (*Cuminum cyminum*)[7]. *Sadikka* and *Vasavasi* (*Myristica fragrance*), *Karabu* (*Syzygium aromaticum*), *Suduru* (*Cuminum cyminum*), *Asamodagam* (*Trachispermum ammi*), *Athividayan* (*Aconitum hetarophyllum*), *Tippili* (*Piper longum*), *Kaluduru* (*Nigella sativa*) and *Katukarosana* (*Picrorrhiza kurroa*) are used as *Kalka dravya* of *Neelyadi* oil.

Materials and methods

Materials

Mueller-Hinton agar, Nutrient broth, Distilled water, Conical Flasks, Petri plates, Cork borer, Chemical balance, Micropipettes.

Methods

Anti-bacterial assay

Microbial assays were conducted using Mueller Hinton Agar medium. An amount of 37.0 g of Mueller Hinton agar and 3.0 g of bacteriological agar were weighed and dissolved in 1 L aliquot of distilled water. The mouth was tightly covered with cotton wool and autoclaved at 121°C for 15 minutes. The medium was poured into sterile culture plates inside the biosafety cabinet. *Neelayadi, Visarpahara,* and *Seethodaka* oils were tested for antibacterial activity against standard-type strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25922). A loopful of culture was added to a 5 mL aliquot of saline and incubated overnight at 37^0C. The suspension was compared with the McFarland standard.

Five wells were made in each plate and the bottom was sealed using molten agar. Tetracycline (1000 ppm, 50µL for Staphylococcus aureus) or Azithromycin (1000 ppm, 50µL for E. coli) and DMSO (10 %, 50 µL) were prepared. An aliquot of 25.00 µL from *Neelayadi* oil, *Visarpahara*, and *Seethodaka* oil was separately placed into the wells by dissolving DMSO (10 %, 25 µL). Tetracycline or Azithromycin and DMSO were used as positive and negative controls, respectively. The zone of inhibition was measured after incubation at 37°C for 24 hours. Each assay was done in triplicate, and the average zone of inhibitions was calculated.

Results

Antibacterial Assay plate description for *S. aureus* is shown in Figure 1.

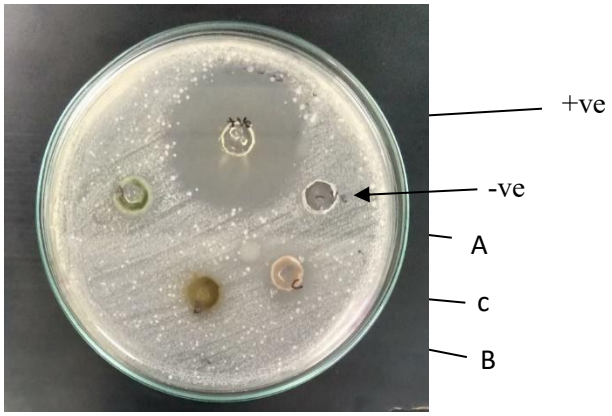

Fig. 1: Antibacterial Assay plate description for S. aureus. (A – *Neelyadi* oil, B- *Seethodaka* oil, C- *Visarpahara* oil)

Table 1 shows the results of the tested oils against *Staphylococcus aureus*

Oil	Test micro organism	Test drug inhibition zone diameter (mm)	Positive control inhibition zone diameter	Negative control inhibition zone diameter
Seethodaka oil	*S. aureus*	12mm	40mm	00mm
Visarpahara oil	*S. aureus*	16mm	40mm	00mm
Neelyadi oil	*S. aureus*	13mm	40mm	00mm

Figure 2 shows the Antibacterial Assay plate description for *E. coli*.

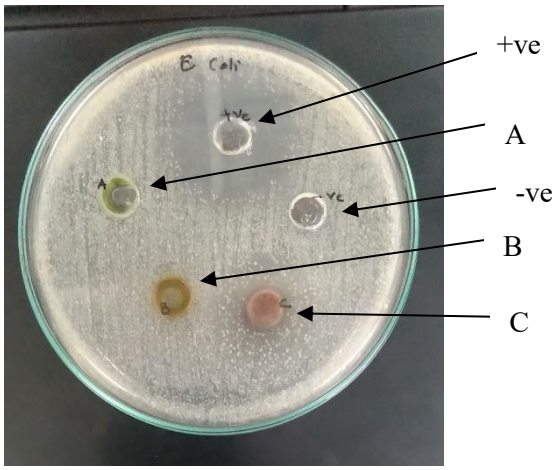

Fig. 2: Antibacterial Assay plate description for *E. coli*. (A –*Neelyadi oil*, B- *Seethodaka oil*, C- *Visarpahara oil*)

The results of the tested oils against *E. coli* are shown in Table 2.

Table 2: Results of the tested oils against *E. coli*

Oil	Test micro organism	Test drug inhibition zone diameter (mm)	Positive control inhibition zone diameter	Negative control inhibition zone diameter
Seethodaka oil	*E .coli*	8mm	30mm	00mm
Visarpahara oil	*E. coli*	24mm	30mm	00mm
Neelyadi oil	*E. coli*	11mm	30mm	00mm

Discussion

The results exhibit an inhibition diameter of 12mm for *Seethodaka* oil against *S. aureus* while 16mm diameter for *Visarpahara* oil and 13mm of diameter for *Neelyadi* oil against the same bacteria. The positive control, tetracycline shows a 40mm inhibition zone diameter against *S. aureus* (Table 1). According to the results, all three oils have a considerable antimicrobial effect against staphylococcus aureus. Staphylococcus aureus is a gram-positive bacterium that is the leading cause of skin and soft tissue infections such as abscesses, furuncles, and cellulitis[11]. Abscess is the pocket of infection and is usually filled with pus and has inflammatory signs such as redness, pain, and warmth. Cellulitis is an infection of underlying layers of the skin that mostly occur in the legs or arms[11]. It also shows inflammatory signs. According to the results, *Seethodaka*, *Visarpahara,* and *Neelyadi* can clinically apply to skin diseases such as Abscesses, Furuncles, and cellulitis due to their antimicrobial activity against *S. aureus*.

According to the results (Table 2), 8mm of inhibition zone diameter shows *f*or *Seethodaka* oil against *E. coli,* and an incredible antimicrobial effect is shown by *Visarpahara* oil against *E. Coli* with an inhibition zone diameter of 24 mm. *Neelyadi* oil also has a considerable inhibition zone diameter (12mm) against *E. coli*. The inhibition zone diameter of positive control against *E. coli* is 30mm. The primary habitat of *E. coli* is the gut but it may survive on skin due to unhygienic conditions as well as skin infections[12]. *E. coli* strains are isolated from skin and skin infections, confirming their presence. The results indicate all three oils have antimicrobial activity against *E. coli* and can apply to skin conditions that lead to generating harmful effects by *E. coli*.

Visarpahara oil has a potent antibacterial effect against both *E. coli* and *S. aureus* compared to the other two oils. The number of mineral ingredients such as sulphur, *Manoshila* (Arsenic sulphide), Copper sulphate, and *Sivanguru* (Fe_2O_3) of *Visarpahara* oil may lead to having an enhanced antibacterial effect than the other two oils. In Ayurveda, *Rasa shasthra* has mentioned a number of mineral compounds for several diseases and those are more effective than herbal compounds. *Gandaka* (sulphur) and its preparations are used for skin diseases for thousands of years[10]. Sulphur has an amazing antibacterial effect against Staphylococcus aureus[13]. It is important in the management of dermatological conditions such as scabies, acne, and dandruff[14].

Conclusion

All tested traditional oils of *Seethodaka, Visarpahara,* and *Neelyadi* have a considerable antibacterial effect against *S. aureus* and *E. coli*. Therefore, all three oils can effectively be used for managing skin conditions caused by *S. aureus* and *E. coli*. Among the tested three oils, *Visarpahara* oil shows the highest inhibition zone diameter against both *S. aureus* and *E. coli* than *Seethodaka* oil and *Neelyadi* oil. *Visarpahara* oil shows the highest inhibition zone diameter for *E. coli* (24mm) than *S. aureus* (16mm). *Seethodaka* oil shows a high inhibition zone diameter for *S. aureus* than *E. coli*. *Neelyadi* oil also shows a better antimicrobial effect against *S. aureus* than *E. coli*.

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Pharmacognostic studies on stem bark of *Canarium zeylanicum* (Retz.)

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Abstract

Canarium zeylanicum (Retz.) of the family Burseraceae, is an endemic medicinal plant of Sri Lanka which is used for medicinal purposes and as a fumigant to repel mosquitoes for ages. Though, it is endemic, a large number of plants are available throughout the wet zone which can be sustainably utilized for the wellbeing of the public. The present study aims to establish data on macroscopical, microscopical, and certain phytochemical and physicochemical characteristics of stem bark and stem bark powder. Three representative samples were collected from three geographical locations of Sri Lanka and then macroscopical, and microscopical characteristics were investigated. Preliminary phytochemical screening was done on the bark powder after the sequential extractions on hexane, dichloromethane (DCM), and methanol, followed by the development of Thin Layer Chromatography (TLC) fingerprints for the above extracts. Total terpenoid content was estimated in the hexane fraction. Physico-chemical characteristics; total ash, acid-insoluble ash, and moisture contents were analysed. The essential oil content of the bark was determined using the Clevenger apparatus. Microscopically, an abundance of groups of pitted lumen stone cells, crystal fibres, and the presence of prismatic crystals of Calcium oxalate were characteristic anatomical features in the cortical region. Alkaloids, saponins and tannins were observed in methanol fraction and terpenoids in hexane and DCM fractions. Total terpenoid content, total ash and acid insoluble ash contents were 391.84±3.98 mg/g, 9.72±0.07%w/w and 5.00±0.05%w/w respectively. Moisture content was 9.96±0.03%v/w on a wet basis and the essential oil content was 0.26±0.01%v/w on a dry weight basis. Data generated by the present study may be useful to determine the quality and purity of the stem bark of Canarium zeylanicum (Retz.).

Keywords: *Canarium zeylanicum* (Retz.), TLC, Burseraceae, Pharmacognosy, Terpenoids

Introduction

The plant *Canarium zeylanicum* (Retz.), a species of flowering plant in the family Burseraceae, is endemic to Sri Lanka. In Sinhala this plant is known as "*Dik Kekuna*" and in Tamil, it is known as *Skillil* (*Pakillipal*)[1].

Canarium zeylanicum (Retz.) is a large branched tree that grows up to 25–30m in height. It has imparipinnate compound leaves, arranged alternatively on the stem branches 1 and green colour flowers, arranged in terminal panicles on short, stout pedicels. The peel of the nut is thick and copper in colour. In the centre of the fruit, there is 1-2 seed covered by white flesh. The seed contains a high-fat white kernel. The bark is about 4mm thick, hard, and pale brown in colour. Gum is naturally occurring in the plant stem and when the bark is injured, an abundance of a beautifully clear, fragrant, balsamic gum resin exudes from it and this is used for fumigation in houses against mosquitoes for ages[2].

Medicinally, the bark of the tree is astringent and antiseptic. A decoction of it is used as a gargle for bleeding and spongy gums. An ointment prepared

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by boiling bark with Coconut oil is used as a dressing for chronic ulcers. Internally, it is used as an aromatic stomachic and astringent and is commonly used against diabetes[2].

Although the stem bark is used for a variety of therapeutic purposes in traditional medicine and as a fumigant against mosquitoes for ages, there is no detailed Pharmacognostic data reported on the bark of *Canarium zeylanicum* (Retz.) plant. Therefore, the present study aims to establish Pharmacognostic data on the stem bark of *Canarium zeylanicum* (Retz.), for its authentication and to develop value-added standardized products with commercial importance, based on the stem bark.

Materials and Methods

Collection of plant materials

Canarium zeylanicum (Retz.) stem barks were collected from matured plants at Bulathsinghala of Kaluthara district, Deiyandara of Matara district, and Dambadeniya of Kurunegala district in Sri Lanka. The authenticity of plant material was accessed by comparing it with local floras available in textbooks at the Pharmacognosy laboratory of the Industrial Technology Institute, Malabe, Sri Lanka.

Macroscopical and microscopical studies

Preparation of samples

For macroscopical and microscopical studies thoroughly washed stem barks were cut into desired sizes (7cm-9cm) and preserved in formaldehyde acetic acid and alcohol solution (FAA). For powder microscopical studies dried stem bark samples were grinded, sieved and 120 mesh powder was obtained and stored in air-tight containers until used for further analysis[3].

Macroscopical analysis

Macroscopic character evaluation of the bark and powder samples were carried out by examining its colour, odour, texture and appearance.

Microscopical analysis

Method of slide preparation

Freehand sections of stem bark were taken using a sharp blade and were mounted with Chloral hydrate solution to observe various anatomical features. Specimen slides, so prepared were observed under mid-power 10x followed by a high-power 40x of "Labomed Sigma" compound microscope. For powder, microscopical identification 120 mesh powder was mounted with Chloral hydrate and observed as earlier[4,5].

Determination of phytochemical characteristics extraction of the samples

Dried bark powder (50g) was sequentially extracted with hexane, dichloromethane, and methanol as solvents by hot Soxhlet extraction method[6].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out for each extract of sequential extractions using standard protocols to identify constituents [7,8,9,10,11].

Development of Thin Layer Chromatography profiles

The sequential extracts obtained from Soxhlet extractions were spotted on pre-coated silica gel 60G F_{254} aluminum plates. Different solvent systems were used for methanol, DCM, and hexane extracts to obtain a clear separation of compounds as Table 1.

Table 1: Development of Thin Layer Chromatography profiles with different solvent systems

Type of extract	Solvent system ratio			
	Methanol(v/v)	DCM (v/v)	Hexane (v/v)	Formic acid (v/v)
Methanol extract	4	3	1	0.5
DCM extract	5	5	1.5	0.5
Hexane extract	5	5	2	0.5

Then TLC profiles were developed and the retention factors (Rf) were calculated at wavelength 254nm, 366 nm, and after spraying vanillin sulphuric[12,4,5].

Determination of Total Terpenoid Content (TTC)

Initially, 10 g of *Canarium zeylanicum* (Retz.) bark powder was subjected to Soxhlet extraction with hexane and bark powder hexane extract was obtained. Then TTC was determined using 300mg of bark powder hexane extract according to standard protocols using Linalool as standard [13,14,15].

Determination of physico-chemical characteristics

Total ash, acid insoluble ash, and moisture content of the powder samples were determined according to standard protocols [16,17,18].

Determination of essential oil content

Initially, 100.00 g of bark powder was subjected to hydro distillation using Clevenger-type apparatus, and essential oil content was determined [19,20].

Results

Macroscopical characters

Canarium zeylanicum (Retz.) stem bark samples were 6 to 7mm in thickness, externally rough, fracture was fibrous and light brown in colour with a characteristic odour. The transverse section of the stem bark was pale brown in colour and the bark powder was light brown with a characteristic aromatic odour.

Microscopical characters

In the cork region hexagonal thin-walled parenchyma cells most filled with brown colouring matter and a few groups of stone cells, thick-walled fibres were also observed.

In the cortical region; an abundant group of stone cells with pitted lumen and large groups of thick-walled fibres were observed. Some fibre cells were associated with prismatic crystals of Calcium oxalate as crystal fibres and it was identified as a characteristic feature of the stem bark. Long fibres and short fibres were abundant and amongst them some were wide-lumen, short fibres. The abundance of isolated prismatic crystals of Calcium oxalate was observed among stone cells and thick-walled fibres. Isolated and compound starch grains were also found in the cortical region and vessels with spiral arrangement and biseriate to multiseriate medullary rays were observed in the cross section of stem bark cortical region of *Canarium zeylanicum* (Retz.) (Plate 1).

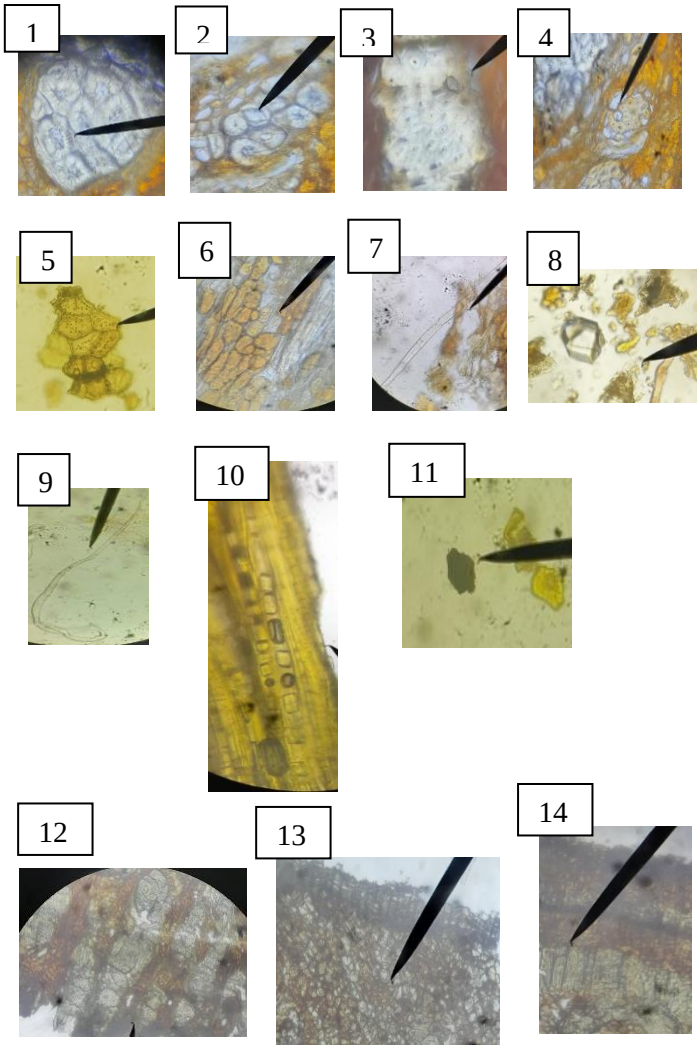

Plate 1: Photographic images of microscopical studies (Labomed Sigma" USA compound microscope)

1) Stone cells 2) Stone cells associated with Calcium oxalate prismatic crystal 3) Thick walled fibres in sectional view 4) Thick walled fibres in sectional view associated with a prismatic crystal 5) Pitted walled fibres in sectional view 6) Essential oil filled cells 7) Wide lumen short fibres 8) Prismatic crystal 9) Long fibres 10) Calcium oxalate prismatic crystals associated fibres 11) Starch grain
12) Transverse section of the cortical region
13) Transverse section of the cork region
14) Transverse section of the bark

Preliminary phytochemical study

Preliminary phytochemical studies revealed the presence of alkaloids, tannins, and saponins in methanol extract and terpenoids in hexane and dichloromethane extract (Table 1).

Table 1: Preliminary phytochemical screening of stem bark powder extract of *Canarium zeylanicum* (Retz.)

Phyto chemicals	Hexane extract	Methanol extract	Dichloromethane extract
Alkaloids	-	+	-
Flavonoids	-	-	-
Tannins	-	+	-
Saponins	-	+	--
Terpenoids	+	-	++
Steroids	-	-	-

++ (Present highly), + (Present), - (Absent)

Thin Layer Chromatography (TLC) profiles of Canarium zeylanicum (Retz.) stem bark powder extracts

Thin Layer Chromatography (TLC) profiles of *Canarium zeylanicum* (Retz.) stem bark powder extracts with DCM extract, Methanol extract and Hexane extract are shown in plate 2(A), 2(B) and 2(C).

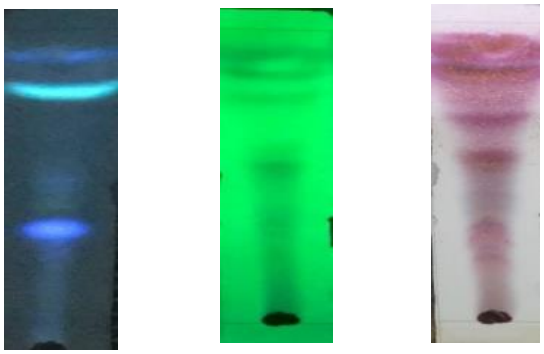

Plate 2(A): TLC fingerprint profile of DCM extract

A1) at 365nm A2) at 254nm A3) After derivatisation using Vanillin sulphuric

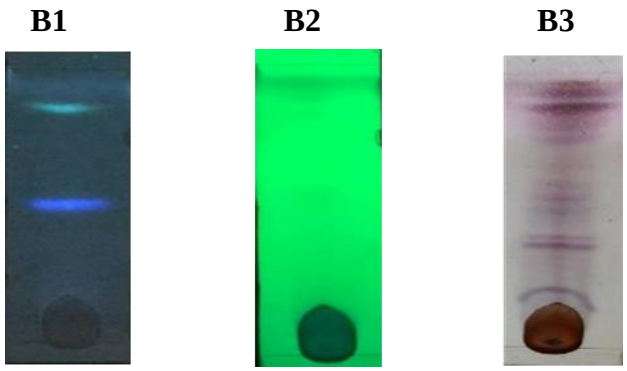

Plate 2(B): TLC fingerprint profile of Methanol extract

B1) at 365nm B2) at 254nm B3) After derivatisation using Vanillin sulphuric

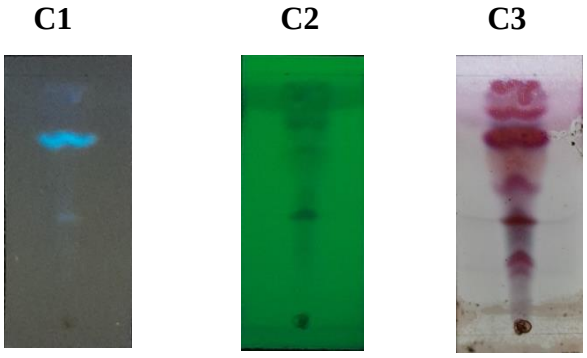

Plate 2(C): TLC fingerprint profile of Hexane extract

C1) at 365nm C2) at 254nm C3) After derivatisation using Vanillin sulphuric

TLC details of Canarium zeylanicum (Retz.) stem bark powder extracts

TLC studies established chromatographic patterns for *Canarium zeylanicum* (Retz.) stem bark powder dichloromethane, methanol, and hexane extracts. After derivatisation using vanillin sulphuric, dichloromethane extract with solvent system DCM, hexane, ethyl acetate, formic acid has shown fifteen coloured spots, methanol extract with solvent system DCM, cyclohexane, ethyl acetate, formic acid has shown twelve coloured spots and hexane extract with solvent system DCM, hexane, ethyl acetate, formic acid has shown twelve coloured spots (Table 2,3 and 4).

Table 2: TLC details of *Canarium zeylanicum* (Retz.) stem bark powder extracts (solvent system DCM)

Dichloromethane (DCM) extract

Rf values at day light	Rf values at UV light		Rf values after derivatisation
	254nm	365nm	
0.92	0.24	0.38	0.19-Brown
	0.29	0.45	0.24-Light
	0.32	0.54	purple
	0.38	0.81	0.26-Pink
	0.50	0.92	0.29-Orange
	0.56		0.35-Yellow
	0.75		0.48-Pink
	0.81		0.49-Blue
	0.92		0.54-Brown
			0.66-Purple
			0.76-Brown
			0.78-Blue
			0.80-Pink
			0.86-Pink
			0.90-Brown
			0.92-Pink

Table 3: TLC details of *Canarium zeylanicum* (Retz.) stem bark powder extracts (solvent system DCM)

Methanolic extract

Rf values at daylight	Rf values at UV light		Rf values after derivatisation
	254nm	365nm	
	0.81	0.50	0.21-Purple
	0.86	0.78	0.75-Purple
	0.92	0.91	0.38-Purple
			0.80-Purple
			0.40-Purple
			0.86-Violet
			0.5-Blue
			0.92-Violet
			0.55-Purple
			0.94-Violet
			0.60-Purple
			0.66-Light brown

Table 4: TLC details of *Canarium zeylanicum* (Retz.) stem bark powder extracts (solvent system DCM)

Hexane extract

Rf values at day light	Rf values at UV light		Rf values after derivatisation
	254nm	365nm	
	0.32	0.32	0.25 Purple
	0.43	0.43	0.28-Pink
	0.73	0.52	0.32-Violet
	0.92	0.68	0.45-Brown
	0.95	0.80	0.46-Blue
		0.83	0.58-Pink
		0.91	0.65-Pink
			0.66-Orange
			0.78-Pink
			0.85-Pink
			0.92-Pink
			0.98-Pink

Physiochemical characteristics, essential oil content and total terpenoid content of stem bark powder of *Canarium zeylanicum* (Retz.)

Physio-chemical characteristic values, essential oil content and total terpenoid content of *Canarium zeylanicum* (Retz.) bark powder is shown in Table 5.

Table 5: Physicochemical values, essential oil content and total terpenoid content of *Canarium zeylanicum* (Retz.) stem bark powder

Parameter	value
Total ash content%(w/w)	9.72±0.07%
Acid insoluble ash content%(w/w)	5.00±0. 0.05%
Moisture content%(w/w)	9.96±0.03%
Essential oil content%(v/w)	0.26±0.01%
Total terpenoid content (TTC)	391.84±3.98mg Linalool equivalent /g

Results are represented as mean ±SE(n=3)

Discussion

The macroscopical and microscopical analysis is one of the cheapest method, that can be used as an identifying parameter to substantiate and authenticate a drug [21]. Therefore, observed macroscopical and microscopical characteristics of stem bark and bark powder would be a useful tool for plant identification.

Phytochemical analysis reveals useful findings about the chemical nature of the stem bark powder. Tannins, saponins, alkaloids, which were found in stem bark extracts are known to have beneficial medicinal properties while terpenoids are known to have anti-inflammatory, antiviral, and insect-repellent activity[15,22,23]. For phytochemical analysis plant extracts were obtained using the Soxhlet extraction method because it makes the extraction process much more efficient with a high extraction yield that requires less time and solvent consumption than maceration or percolation [24]. First, bark powder was extracted with nonpolar hexane as the solvent, then with dichloromethane as the solvent and later with the methanol according to the polarity increasing order resulting in sequential extraction. Sequential extraction ensures the extraction of all active components according to their polarity order [6]. Thin Layer Chromatography (TLC) is an affinity-based technique used to separate the constituents of a mixture [25]. Results of TLC fingerprint profiling can be used as a quality standard for purity determination of Canarium zeylanicum (Retz.) bark powder.

Physicochemical parameter determination is an important criterion to judge the identity or purity of crude drugs. Ash contents reveal how much minerals are physiologically contained in the medicinal plants and how many foreign materials have been mixed in during the course of processing [16]. According to the obtained data total ash content was within the average range compared to other plants. But acid insoluble ash content has much higher values and can be assumed that, it is due to contamination of bark powder with silica crystals. The moisture content was determined using Dean and Stark method because bark powder contains volatile oils which makes the conventional oven drying method not applicable. As obtained results moisture content of the bark was within the acceptable range of 8 - 12% for minimum bacteria, and fungal growth [26].

The essential oil yield of plants is often below 1%, rarely reaching 10% or even more in some dry plant parts. The plants which contain at least 0.1%-0.2% volatile oil content with a perceptible odour is considered as economically viable aromatic plants. The essential oil content of Canarium zeylanicum (Retz.) bark powder is within the range and can be used to develop value-added aromatic products.

Conclusion

Through this study, identification data of *Canarium zeylanicum* (Retz.) stem bark and bark powder were established based on macroscopical and microscopical characteristics. Quality standards for *Canarium zeylanicum* (Retz.) stem bark powder has been established with respect to certain physicochemical and phytochemical characteristics. The data revealed in this study can be used as a reference to determine quality and the purity of *Canarium zeylanicum* (Retz.) stem bark in future studies and for the development of herbal-based quality products.

Acknowledgement

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Scientific review on *Katukaskandhaya* (group of pungent drugs) and its medicinal importance

Narathota S.N.L.[1*] and Jayasiri A.P.A.[2]

Abstract

Dravyaguna vinjnana of Ayurveda which can be correlated with modern Pharmacology, is an area with a wide spectrum of knowledge regarding substances that are used in treatment and preventive care. The study of *Dravya* with their properties and actions helps in the proper utilization of drugs for different diseases. Classification based on the dominancy of taste (*Rasa*) is mentioned under the term *Skandha* in *Charaka Samhitha* and drugs with pungent taste come under the *Katukaskandhaya*. In this scientific review, ten drugs mentioned in *Katukaskandhaya* were evaluated based on *Rasadi panchakaya*, chemical composition and therapeutic efficacy with reference to Ayurveda, Traditional, Allopathic medical texts and research articles published in validated databases. Among the selected drugs, majority (>60%) were having *Laghu*, *Ruksha*, *Thekshna Guna*, 80% with *Ushna Veerya* and 70% with *Katu Vipaka*. These drugs were prominent with Alkaloids, Terpenes, Phenols, and Flavonoids recorded with Antioxidant, Anti-microbial and Anti-carcinogenic properties. According to therapeutic effectiveness and efficacy mentioned in Ayurveda and Traditional medical texts, these are mainly indicated for the treatment of diseases caused due to vitiated *Kapha-Vata dosha*. Vitiation of *Kapha dosha* leads to impairment of *Agni* resulting formation of *Ama* which is a causative factor for many disorders. *Trikatu* is one of the drug formulae mentioned for vitiated *Kapha dosha* which includes drugs mentioned in *Katukaskandhaya* such as *Zingiber officinale, Piper longum* and *Piper nigrum*. Further studies on this group of drugs can be conducted with phytochemical analysis and clinical trials to analyze the efficacy of drug formulae with drugs of *Katukaskandhaya* in disease management.

Keywords: *Katukaskandhaya*, Pungent taste, *Ushna veerya*, Chemical composition, Pharmacological actions

Introduction

Ayurveda is an Indian philosophy that mainly focuses on maintaining good health by balancing physical, mental and spiritual well-being in order to treat and get prevented from diseases[1]. This is a medical field of holistic approach with use of herbal medicines. *Dravyaguna Vignana*[2] is the area that mainly focuses on the *Guna* (properties) and *Karma* (actions) of herbal materials. This can be correlated with modern pharmacology and proper understanding of this area is advantageous in selecting and prescribing suitable medicines for different disease conditions. In Ayurveda, *Panchamahabhuta*[3] (*Akasha, Vayu, Agni, Jala, Prithivi*) are regarded as physicochemical basis of the materials and *Tridosha*[4] (*Vata, Pitta, Kapha*). *Tridosha* have specific functions of movement, assimilation and growth respectively which have derived from the qualities of these *Panchamahabhuta*.

According to Charaka Samhitha, both the *Guna* and *Karma* reside or are in concomitant with *Dravya*[5]. Five properties residing in *Dravya* are mentioned in

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Bhavaprakasha[2] and they are, *Rasa* (taste), *Guna* (properties), *Veerya* (potency), *Vipaka* (final transformed state after digestion) and *Prabhava* (special potency). The word *Rasa* can be attributed in different contexts such as, *Rasa Dhatu* which circulates all over the body, *Parada* (Mercury) mentioned in Alchemy and the taste that can be perceived through taste buds[6]. There are six tastes mentioned in Ayurveda which is known as *Shadrasa*. They are, *Madhura* (sweet), *Amla* (sour), *Lavana* (salty), *Katu* (pungent), *Tikta* (bitter) and *Kashaya* (astringent)[7]. Knowledge about the classification of *Dravya* in Ayurveda is important in prescribing the best drug for a disease. Classification based on the dominancy of taste (*Rasa*) is mentioned under the term *Skandha* in Charaka Samhitha[7] and drugs with pungent taste come under the *Katukaskandhaya*[8]. This group of drugs is commonly present with *Katu rasa* (pungent taste), *Laghu*, *Ruksha*, *Theekshna guna* (light, rough, sharp properties) and hot potency (*Ushna veerya*).

The *Katu rasa* or the pungent taste is made with *Vayu* (air) and *Agni* (fire) elements[5] and is present with hotness, lightness, dryness and sharpness[9]. These properties help in balancing and reducing vitiated *Kapha dosha* while slightly increasing *Pitta dosha* due to sharp property (*Theekshna guna*). Hot potency (*Ushna veerya*) helps in pacifying both *Kapha* and *Vata dosha*[10]. A pungent taste is said to be felt by the central region of the tongue and blood, and reproductive tissues are said to be most affected by this taste[6]. There are three *Vipaka* (final transformed states after digestion) mentioned in Ayurveda as *Madhura vipaka* (sweet), *Amla vipaka* (sour) and *Katu vipaka* (pungent). Pungent taste conversion is considered as *Katu vipaka* and food or medicines that are with sharp, hot, dry final transformation after digestion can be classified under *Katu vipaka*[11]. The majority of the drugs of *Katukaskandhaya* are present with *Katu vipaka* (final transformed state after digestion) which is one of the three *Vipaka* mentioned in Ayurveda.

Pungent taste is said to be having carminative, diaphoretic, and vasodilatory properties. Vegetables, radishes, turnips, raw spinach, leeks, onions, garlic, Grains, Nuts, Seeds, Mustard seeds, most spices, especially black pepper, cardamom, cloves, ginger are some examples for food with pungent taste[6]. In Susrutha Samhitha, *Katu rasa* (Acrid taste) is mentioned as an appetizer, and digestive agent, that eliminates *Dosha*, and alleviates obesity, laziness, expectoration, worms and poisons. It also mentions that the excess can cause negative effects like intoxication, dryness, giddiness and burning sensation as well[12].

In Ayurveda, *Katukaskandhaya* is the category consisting of drugs having a Pungent taste. There are 39[13] drugs mentioned in this classification and the *Surasadi*[14] and *Pippalyadi gana*[15] mentioned in Susrutha Samhitha are also included in this *Katuka* group of drugs. Many of the drug recipes used by Ayurveda and Traditional medical practitioners include these drugs mentioned under *Katukaskandhaya*. Commonly these drugs have been used in treating diseases caused by *Agnimandya* that occurred due to malfunctioning of *Kapha dosha*. Other than that, drug recipes have been mentioned for the management of diseases caused by *Kapha-Vata* origin as well.

The general objective of the study was to provide a clear and comprehensive analysis of the pharmacological actions and medicinal importance of *Katukaskandhaya*. Specific objectives were to analyze the chemical nature, therapeutic indications, benefits, and importance of these drugs with reference to Ayurveda, the Traditional medical system and the Allopathic system of medicine.

Materials and Methods

This review was conducted with refence to Ayurveda authentic texts such as Charaka Samhitha, Susrutha Samhitha, Bhaisajya Rathnavali, Sharangadhara Samhitha, Dravya Guna Vignana, A text book of Dravyaguna Vignana and Dravya Muladharma. Traditional medical texts like Vaidyaka Sarasankshepaya, Kashaaya Sangrahaya, Kashaya Sagaraya, Ayurveda Aushada Sangrahaya were also referred to in order to check the applications, indications and analysis on *Rasadi panchakaya* of this group of drugs mentioned under

the *Katukaskandhaya*. Published research articles, Journal articles from valid websites and databases like PubMed, Google Scholar and other sources were also used to collect information about the chemical nature and medicinal importance of the drugs mentioned under the *Katukaskandhaya*.

In the present study, the pharmacological properties, therapeutic indications and chemical nature of 10 selected drugs mentioned under the *Katukaskandhaya* were analyzed. Ten drugs of *Katukaskandhaya* were selected based on their utility in the therapeutic aspect. Selected drugs have been included in many of the drug formulae commonly used in the management of diseases caused due to vitiated *Kapha* and *Vata doosha*. *Piper longum* L. (Long Pepper), *Piper nigrum* L. (Pepper), *Zingiber officinale* Roscoe. (Ginger), *Plumbago zeylanica* L. (Wild White Leadwort/ Ceylon Leadwort), *Elettaria cardamomum* L. (Cardamom), *Apium graveolens* L., (Wild Celery), *Brassica alba Boiss* L. (White Mustard), *Ferula assafoetida* L. (Asafoetida), *Embelia ribes* L. (False-black pepper) and *Allium sativum* L. (Garlic) were the ten selected drugs. A comprehensive analysis was conducted with reference to the above-mentioned sources regarding the pharmacological aspects, medicinal importance and the chemical nature of the selected set of drugs mentioned in *Katukaskandhaya* of Ayurveda.

Results and Discussion

According to *Charaka Samhitha*, drugs having similar *Rasa*, *Guna*, *Veerya*, *Vipaka* and *Prabhava* are categorized under 6 *Skandhas*, which are named according to the 6 tastes mentioned in Ayurveda[8]. It has stated that *Katukaskandhaya* contains 39 drugs [13]. Some drugs mentioned in *Surasadi gana* [14] which contains 21 drugs and *Pippalyadi gana* which contains 22 drugs[15] have also been included in *Katukaskandhaya*. From the drugs and other ingredients mentioned in the text, some of the ingredients cannot be identified and cannot be seen today. Therefore, this literature study was conducted based on 10 drugs mentioned under the *Katukaskandhaya* which can be clearly identified and commonly used today.

Among the drugs mentioned in *Pippalyadi gana*, 13 are mentioned in the *Katukaskandhaya* as well. They are, *Pippali, Pippali mūla, Chavya, Chithraka*, *Nagara*, *Maricha*, *Gaja pippali*, *Harenuka*, *Ela*, *Ajamoda, Sarshapa, Hingu* and *Vidanga* [15]. Both the dried and fresh ginger (*Zingiber officinale* Roscoe) has been mentioned in *Katukaskandhaya,*[13] but fresh ginger (*Ardraka*) has not been mentioned in the *Pippalyadi gana*. Most of the drugs mentioned in *Surasadi gana* are unknown and 8-9 drugs are called as varieties of *Tulasi* or appear to be different species of the family LABIATEAE (LAMIACEAE)[16]. *Vidanga* is mentioned in all these three groups of drugs.

Other than the herbal drugs mentioned in the classification, different materials and constituents like *Mutra* (urine), *Kshara* (alkaline) and *Pitta* (bile) have also been mentioned in *Katukaskandhaya*. In identifying and describing the pungent taste, it has been described as an alkaline taste in some of the authentic texts. In order to analyze the pharmacological features and functions on body tissues, *Panchapadartha* or the *Rasadi panchakaya* [17] was also analyzed. Term *Panchapadartha* has been introduced by Acharya Bhavamisra, in his text named, Bhavaprakasha[2].

According to the analysis of these ingredients (Table 1 and Figure 1), it was clear that among the selected 10 drugs, 2 drugs (13.3%) belong to the family PIPERACEAE, two drugs belong to the ZINGIBERACEAE family (13.3%) and two drugs belong to the APIACEAE or the UMBELLIFERAE family [18].

PIPERACEAE, the pepper family in the order Piper ales is commercially important because of *Piper nigrum*, the source of black and white pepper. The family comprises about 5 genera, of which Piper (about 2,000 species) and Peperomia (about 1,600 species) are the most important. The plants grow as herbs, vines, shrubs, and trees and are widely distributed throughout the tropics and subtropics[19].

Table 1: Nomenclature and details of the selected ten drugs [17]

	Botanical name and English name	Family name	Sanskrit name	Sinhala name	Used part
1	*Piper longum* L. (Long Pepper)	PIPERACEAE	*Pippali*	*Thippili*	Fruits
2	*Piper nigrum* L. (Pepper)	PIPERACEAE	*Maricha*	*Gammiris*	Seeds
3	*Zingiber officinale* Roscoe (Dried Ginger)	ZINGIBERACEAE	*Nagara*	*Wiyali Inguru*	Rhizome
	Zingiber officinale Roscoe (Fresh Ginger)		*Ardraka*	*Amu inguru*	
4	*Plumbago zeylanica* L. (Wild White Leadwort/ Ceylon Leadwort)	PLUMBAGINACEAE	*Chithraka*	*Elanitul*	Roots, Stem
5	*Elettaria cardamomum* L. (Cardamom)	ZINGIBERACEAE	*Ela*	*Enasahal*	Seeds
6	*Apium graveolens* L. (Wild Celery)	APIACEAE [18]	*Ajamoda*	*Asamodagam*	Seeds
7	*Brassica alba Boiss* L. (White Mustard)	BRASSICACEAE	*Sarshapa*	*Ela Aba*	Seeds
8	*Ferula assafoetida* L. (Asafoetida)	APIACEAE	*Hingu*	*Perumkayam*	Resin
9	*Embelia ribes* L. (False-black pepper)	PRIMULACEAE	*Vidanga*	*Walanga sahal*	Seeds
10	*Allium sativum* L. (Garlic)	AMARYLLIDACEAE	*Lasuna*	*Sudu lunu*	Bulbs

Fig. 1: Selected Ten drugs of *Katukaskandhaya*

ZINGIBERACEAE commonly known as the ginger family, is a family of flowering plants comprising more than 1300 species divided into about 52 genera of aromatic perennial herbs with creeping horizontal or tuberous rhizomes, distributed throughout tropical Africa, Asia, and America[20]. The APIACEAE or UMBELLIFERAE, commonly known as the celery, carrot, or parsley family, is a family of mostly aromatic plants with hollow stems. Many plants of this family are condiments or vegetables with some of them having medicinal properties[18].

Specific features of these drugs can be understood by studying the *Rasadi panchakaya*[2,21] which includes *Rasa, Guna, Veerya, Vipaka* and *Prabhava.* The study of pharmacology is important in understanding the pharmacological features, and actions of medicines with the integrated knowledge from multiple scientific disciplines including chemistry, biochemistry, molecular biology and physiology, providing a significant positive impact on human health. The scientific knowledge gained through pharmacological studies provides a foundation for a number of medical treatments[22]. In Ayurveda, this analysis helps in understanding the specific actions of drugs on body tissues and systems.

There are six tastes mentioned in Ayurveda which is known as *Shad rasa* such as *Madhura, Amla, Lavana, Katu, Thiktha, Kashaya*[7] and there are 41 *Guna* (properties) of *Dravya*. Among these, 20 *Gurvadi guna*[2] are important in analyzing the pharmacological actions of drugs. Mainly there are 2 types of *Veerya* (potencies) as *Ushna* (hot), *Sheetha* (cold) and three types of final state after digestion/transformation as *Madhura* (sweet), *Amla* (sour) and *Katu* (pungent) *Vipaka*. The special potency of drugs is known as *Prabhava* and this is not commonly seen in most drugs. The following table (Table 2) describes the *Pancha Padartha*[2] mentioned in Ayurveda of the ten selected drugs of *Katukaskandhaya*.

Analysis of the Ayurveda pharmacological properties of selected ten drugs revealed that all the selected drugs were present with *Katu rasa* (100%). 80% of the ingredients were having *Laghu guna,* 90% were having *Theekshna guna,* and 70% were having *Ruksha guna. Ushna veerya* was present in most of the drugs (80%) and dried ginger was recorded to have hot potency whereas fresh ginger was reported to have cold potency. *Piper longum* L. was reported as *Anushna veerya* and it was present with the *Madhura vipaka* as well[20]. 70% of drugs having *Katu vipaka* and *Madhura vipaka* was reported in three drugs including *Piper longum* L., *Zingiber officinale* Roscoe. and *Elettaria cardamomum* L. *Embelia ribes* L. (*Vidanga*) is the only drug that was reported with a special potency and *Krimighna Prabhava*[23] (Anti-helminthic activity) has been recorded in it.

Effects on *Dosha* and effects on five body systems were also analyzed. They were Central Nervous System (CNS), Gastrointestinal Tract (GIT), Cardiovascular System (CVS), Respiratory System (RS) and Genitourinary System (GUS). Common therapeutic indications of these drugs were also analyzed based on the actions of the *Dosha* and its effects on body systems (Table 3).

Analysis of Effects on *Dosha*, body systems and therapeutic indications it was showed that 90% of the selected drugs of *Katukaskandhaya* were having *Kapha shamaka* and *Kapha nashaka guna* which means the ability to pacify vitiated or aggravated *Kapha dosha.* This may be due to the presence of properties like *Laghu* (light), *Ruksha* (rough) which are opposite to the properties of *Kapha dosha.* 90% showed the *Vata shamaka* properties which may be due to the presence of hot potency. 60% of the selected 10 drugs showed *Pitta vardhaka* properties as well. This may be due to the presence of *Theekshna* (sharp/penetrating) property and *Elettaria cardamomum* L. showed *Tridoshahara* properties.

These drugs with the ability in pacifying *Kapha – Vata dosha* are present with beneficial actions on GIT such as *Deepana, Pachana* activities which may be due to the presence of *Katu rasa, Laghu, Ruksha guna* and *Katu vipaka.*

Table 2: *Rasadi Panchakaya* of selected 10 drugs of *Katukaskandhaya* [21]

	Drug	*Rasa* (Taste)	*Guna* (Properties)	*Veerya* (Potency)	*Vipaka* (Taste of final digested product)	*Prabhava* (Special potency)
1	*Piper longum* L.	*Katu* (Pungent)	*Laghu* (Light), *Snigdha* (Oily), *Theekshna* (Sharp/ Penetrating)	*Anushna* (Mild hot potency)	*Madhura* (Sweet)	-----
2	*Piper nigrum* L.	*Katu* (Pungent), *Thiktha* (Bitter)	*Laghu* (Light), *Rūksha* (Rough), *Theekshna* (Sharp/ Penetrating)	*Ushna* (Hot potency)	*Katu* (Pungent)	-----
3	*Zingiber officinale* Roscoe. (Dried)	*Katu* (Pungent)	*Laghu* (Light), *Snigdha* (Oily)	*Ushna* (Hot potency)	*Madhura* (Sweet)	-----
	Zingiber officinale Roscoe. (Fresh)		*Guru* (Heavy), *Ruksha* (Rough), *Theekshna* (Sharp/ Penetrating)	*Sheetha* (Cold potency)		
4	*Plumbago zeylanica* L.	*Katu* (Pungent)	*Laghu* (Light), *Ruksha* (Rough), *Theekshna* (Sharp/ Penetrating)	*Ushna* (Hot potency)	*Katu* (Pungent)	-----
5	*Elettaria cardamomum* L.	*Katu* (Pungent), *Madhura* (Sweet)	*Laghu* (Light), *Ruksha* (Rough)	*Sheetha* (Cold potency)	*Madhura* (Sweet)	-----
6	*Apium graveolens* L.	*Katu* (Pungent), *Thiktha* (Bitter)	*Laghu* (Light), *Ruksha* (Rough) *Theekshna* (Sharp/ Penetrating)	*Ushna* (Hot potency)	*Katu* (Pungent)	-----
7	*Brassica alba Boiss* L.	*Katu* (Pungent)	*Laghu* (Light), *Theekshna* (Sharp/ Penetrating)	*Ushna* (Hot potency)	*Katu* (Pungent)	-----
8	*Ferula assafoetida* L.	*Katu* (Pungent), *Thiktha* (Bitter)	*Theekshna* (Sharp/ Penetrating), *Rūksha* (Rough), *Snigdha* (Oily)	*Ushna* (Hot potency)	*Katu* (Pungent)	-----
9	*Embelia ribes* L. [23]	*Katu* (Pungent), *Kashaya* (Astringent)	*Laghu* (Light), *Ruksha* (Rough), *Theekshna* (Sharp/ Penetrating)	*Ushna* (Hot potency)	*Katu* (Pungent)	*Krimighna*
10	*Allium sativum* L.	*Katu* (Pungent)	*Snigdha* (Oily), *Theekshna* (Sharp/Penetrating), *Pichchila* (Slimy), *Guru* (Heavy), *Sara* (Fluidity)	*Ushna* (Hot potency)	*Katu* (Pungent)	-----

Table 3: Effects on *Dosha*, body systems and therapeutic indications [21]

	Drug (Botanical name)	Effect on *Dosha*	Effect on body systems	Therapeutic indications
1	*Piper longum* L.	*Kapha-Vata shamaka, Pitta vardhaka*	**CNS** – *Medhya,* **GIT** – *Deepana, Thrupthighna, Vatanulomana, Shula prashamana, Mrudu virechana, Krimighna* **CVS** – *Rakthawardhaka, Hrida-utthejaka* **RS** – *Kaphagna* **GUS** – *Vrushya, Muthrala*	*Masthishka-daurbalya, Vataroga, Aruchi Ajeerna, Agnimandya, Vibandha, Arshas, Daurbalya, Pāndu, Amavātha, Shvasa, Kasa, Hikka, Shukra-dhaurbalya*
2	*Piper nigrum* L.	*Kapha-Vata nashaka*	**CNS** – *Utthejaka, Balya* **GIT** – *Lalasrava janaka, Deepana, Pachana, Anulomana, Krimighna, Yakruth- utthejaka* **CVS** – *Utthejaka* **RS** – *Kaphaghna, Kaphanissaraka* **GUS** – *Muthrala, Arthavajanaka*	*Vataja roga, Agnimandya, Ajeerna, Adhmana, Krimi roga, Yakruth vikara, Shvasa, Kasa, Prathishya, Muthrakruchcha, Rajorodha*
3	*Zingiber officinale* Roscoe.	*Kapha-Vata shamaka*	**CNS** – Stimulant, *Vata shamaka* **GIT** – *Thrupthighna, Rochana, Deepana, Pachana, Vatanulomana, Shula prashamana* **CVS** – *Raktha shodhaka, Shothahara* **RS** – *Kaphaghna, Shvasahara* **GUS** – *Vrushya, Vajeekarana*	*Vataja roga, Aruchi, Hrillasa, Ajeerna, Agnimaandya, Adhmana, Kamala, Shvasa, Kasa, Hikka, Prathishya*
4	*Plumbago zeylanica* L.	*Kapha-Vata shamaka, Pitta vardhaka*	**CNS** - In moderate doses, it acts as a stimulant and in excess acts as a toxin **GIT** – *Deepana, Pachana, Krimighna* **CVS** – *Raktha-pitta kopaka, Shothahara* **RS** – *Kaphaghna, Kantaghna* **GUS** - *Garbhasha shasankochaka, Vajeekarana*	*Vataja roga, Agnimandya, Ajeerna, Udara shula, Arshas, Grahani, Yakruth roga, Prathishya, Kasa, Muthrakruchcha, Rajorodha,*
5	*Elettaria cardamomum* L.	*Tridōshahara*	**CNS** – --- **GIT** – *Mukha shodhana, Durgandha nashaka, Chardhi, Thrushna nigrahana, Rochana, Deepana, Pāchana, Anulomana,* **CVS** – *Hridya* **RS** – *Kapha nissaraka* **GUS** – *Muthrajanana*	*Tridoshaja roga, Hriddaurbalya, Vamana, Mukha roga, Hrillasa, Thrushna, Aruchi, Agnimandya, Adhmana, Arshas, Shvasa, Kasa, Muthrakruchcha*
6	*Apium graveolens* L.	*Kapha-Vata shamaka, Pitta vardhaka*	**CNS** – *Masthishka balakaraka* **GIT** – *Deepana, Vatanulomana, Krimighna, Shula prashamana* **CVS** – *Hridaya-Utthejaka* **RS** – *Kaphaghna* **GUS** – *Garbha utthejaka, Muthra pravarthaka, Vajeekarana*	*Vamana, Agnimandya, Adhmana, Udarashula, Krimiroga, Shvasa, Kasa, Hikka, Muthrashula, Muthraghatha, Kashtarthava*

7	*Brassica alba Boiss* L.	*Kapha-Vata nashaka, Pitta vardhaka*	**CNS** – *Vata shamaka,* **GIT** – *Deepana, Vidahi, Krimighna* **CVS** – *Hridaya-utthejaka* **RS** – *Kaphahara* **GUS** – *Garbhasha utthejaka, Vaajeekarana, Muthrakaraka*	*Ardhitha, Pakshaghatha, Sandhivata, Katee shula, Agnimandya, Krimiroga, Shvasa, Kasa, Hikka, Muthrashula, Muthraghatha, Kashtarthava*
8	*Ferula assafoetida* L.	*Kapha-Vata nashaka, Pitta vardhaka*	**CNS** – *Uttejaka, Vedanasthapana, Sanjasthapana, Akshepahara* **GIT** – *Deepana, Pachana, Rochana, Vatanulomana, Shula prashamana, Krimighna* **CVS** – *Hridya* **RS** – *Kapha nissaraka, Shvasahara* **GUS** – *Muthrajanaka, Arthavajanaka, Vajeekaraka*	*Pakshaghatha, Ardhitha, Gudrasi, Akshepa, Agnimandya, Adhmana, Gulma, Krimi roga, Shvasa, Kasa, Hikka, Muthrashula, Vasthishula, Muthrala, Kashtarthava*
9	*Embelia ribes* L. [23]	*Kapha-Vata shamaka*	**CNS** – *Vata shamaka,* **GIT** – *Deepana, Vidahi, Krimighna* **CVS** – --- **RS** – *Kasahara* **GUS** – *Garbhasha utthejaka, Vajeekarana, Muthrakaraka*	*Agnimaandya, Krimiroga, Shvasa, Kasa, Muthrashuula, Muthraghatha, Kashtarthava*
10	*Allium sativum* L.	*Kapha-Vata shamaka, Pitta vardhaka*	**CNS** – *Uttejaka, Vedanasthapana, Medhya* **GIT** – *Deepana, Paachana, Anulomana, Shula prashamana, Krimighna, Yakruth - Uttejaka* **CVS** – *Shothahara* **RS** – *Kapha nissaraka, Khantya* **GUS** – *Muthrajanaka, Shukrajanaka, Arthavajanaka*	*Sandhivata, Gudrasi, Ardhitha, Pakshaghaatha, Urusthambha, Agnimandya, Aruchi, Ajeerna, Krimi roga, Gulma, Hrida roga, Shvasa, Kasa, Muthrakruchcha, Shukradosha, Kashtarthava*

All these features can be directed to the *Pancha-bhautic* nature of the drugs in the *Katukaskandhaya. Shula prashamana* is another action on GIT which may be due to the presence of *Ushna veerya* of ingredients that helps in pain relief by pacifying *Vata dosha*. *Utthejaka, Vedanasthapana, Sanjasthapana* properties can also be seen as the actions of the Central Nervous System. In the Respiratory system, *Kaphaghna, Kaphanissaraka* actions were also reported. These drugs of *Katukaskandhaya* were prominent with *Hridya* and *Shothahara* actions on the Cardiovascular system. *Vajeekarana* (aphrodisiac) effects were also reported in most of these drugs.

According to Ayurveda, *Agni* is considered one of the important concepts and malfunctioning of *Agni* results in the formation of *Ama* which causes diseases [24]. *Agnimandya* is caused due to the reduced function of *Agni* and multiple diseases like *Ajeerna* (indigestion), *Aruchi* (anorexia), *Anaha* (flatulence), *Adhmana* (bloating) are caused due to this. *Samagni* is considered as the best type of *Agni.* Drugs mentioned under the *Katukaskandhaya* are prominent with *Kapha-vata shamaka guna* and it's effective in pacifying and balancing the vitiated *Kapha-vata dosha* and normalizing the function of *Agni.*

Drug groups such as *Deepaneeya Dashakaya, Pippalyadi ghanaya*[25] include many of the drugs mentioned in *Katukaskandhaya* and drug formulae such as *Trikatu,* and *Panchakola*[25] also comprise the drugs mentioned in the *Katukaskandhaya.* These are effective in the management of diseases caused due to vitiated *Kapha dosha*. Traditional texts like *Vaidyaka Sarasanksheepaya*[26] and *Kashaya*

Sagaraya,[25] mention various drug recipes indicated for *Agnimandya* and other disorders caused due to vitiated *Kapha dosha*. Many of these drug recipes include drugs mentioned in the *Katukaskandhaya*.

The chemical nature of the selected 10 drugs was also evaluated in this study and the following table shows the commonly found chemical compositions and their therapeutic effects (Table 4).

The pungent taste is stimulating, invigorating and penetrating, making it an effective way to clear areas of excess moisture that built up stagnation. It also increases circulation, encourages sweating, cleanses blood, muscles and opens internal channels[10]. The pungent properties of medicines, herbs, and spices may be due to the presence of aromatic volatile oils, resins and phytochemicals like glycosides which

Table 4: Analysis of chemical composition and pharmacological actions of selected ten drugs

	Drug (Botanical Name)	Chemical composition	Pharmacological action
1	*Piper longum* L.	1-Piperoylpiperidine (Piperine) is present.	Antioxidant, Anti-inflammatory, immunomodulatory, Anti-asthmatic, Anti-convulsant, Anti-mycobacterial, Anti-amoebic and Anti-cancer activities [27].
2	*Piper nigrum* L.	Terpenoids like α-pinene, β-Pinene, δ3-Carene, Limonene, α-Terpinene-4-ol, p-Cymene. Alkaloids like Piperine [28].	Antioxidant, Antimicrobial
3	*Zingiber officinale* Roscoe	Phenolic compounds like Gingerols, Shogaols, Terpenes, Polysaccharides, Lipids, Organic acids [29].	Antioxidant, Anti-inflammatory, Antimicrobial, Anti-cancer, Neuroprotective, Cardiovascular protective, Respiratory protective, Anti-obesity, Anti-diabetic, Anti-nausea, and Anti-emetic activities.
4	*Plumbago zeylanica* L.	Naphthoquinones, Flavonoids, Alkaloids, Glycosides, Steroids, Tri-terpenoids, Tannins. Plumbagin acid is the most potent and rest are, β sitosterol, 2, 2-dimethyl-5-hydroxy-6-acetylchromene, Sapo-naretin, isoaffinetin [30].	Anti-cancer, Anti-diabetic, Anti-malarial, Anti-microbial activities. Used in Rheumatoid arthritis, Dysmenorrhea, Injury and Cancer [31].
5	*Elettaria cardamomum* L.	α-Terpinol acetate (54.46%) [32]. 1,8-Cineole (20.66 %), Camphene (18.09 %), Camphor (10.02 %), Tricyclene (7.36 %) [33].	Antioxidant Activities
6	*Apium graveolens* L.	Glycosides, Steroide, Furanocoumarin, Flavones, K, Ca and Iron [34]. Caffeic acid, Chlorogenic acid, Apiin, Apigenin, Rutaretin, Ocimene, Bergapten, Seslin, Isoimperatorin, Osthenol, Gravebioside A and B, Umbelliferone [35].	Antibacterial, Antioxidant, Analgesic, Anti-inflammatory and Cytotoxic activities [35].
7	*Brassica alba Boiss* L.	Phenols, Polyphenols, Phenolic acids, Flavonoids, Carotenoids (zeaxanthin, lutein, β-carotene), Alkaloids, Phytosterols Chlorophyll, Glycosylates, Terpenoids, Glycosides [36]..	Antioxidant potential in terms of Metal reducing, Metal chelating, Lipid reducing and Free radical scavenging activities [36].
8	*Ferula assafoetida* L.	It consists of three main fractions, including Resin (40–64%), Gum (25%) and Essential oil (10–17%) [37]. 68% of Carbohydrates, 4% Protein, 1% of Fat, 7% of Minerals, 4% of Fiber.	Relaxant, Memory enhancing, Digestive enzyme, Antioxidant, Antispasmodic, Hepato-protective, Antimicrobial, Anticarcinogenic, Anti-helminthic Antagonistic effect [37].

9	*Embelia ribes* L.	Rich in Essential oils, Alkaloids, Flavonoids, Steroids, Phenolics [38].	Antioxidant activity, Wound healing, Antidiabetic, CNS related disease, Antifertility activity, Antiviral, Anti-obesity, Cardio-protective, Antifungal, Antibacterial [38].
10	*Allium sativum* L.	Rich in Sulfur containing Phyto constituents such as Alliin, Allicin, Ajoene Vinyl dithiins, and Flavonoids such as Quercetin [39].	Anticarcinogenic, Antioxidant, Antidiabetic, Reno-protective, Anti-atherosclerotic, Antibacterial, Antifungal, and Antihypertensive [39].

stimulate the tissues and nerve endings of the mouth with a sensation of sharp and fiery heat. From this analysis, it was clearly understood that most of the selected drugs are present with Anti-microbial, Antioxidant, Anti-inflammatory, Anti-carcinogenic activities along with Free radical scavenging activities. Pungent taste is said to be having effects on warming the body, cleansing, clarifying the sense organs, enhancing digestive fire (*Agni*) and improving digestion, absorption and elimination [40]. *Embelia ribes* L. (*Vidanga*) is well known for its anti-helminthic/ Anti-microbial action (*Krimighna*) [23] and Ayurveda mentions this property as a special potency known as the *Prabhava.* This may be due to the presence of Phytochemicals such as Flavonoids, Phenolic compounds and Alkaloids.

In comparison with modern literature on the chemical nature and medicinal importance of these drugs, it was found that many were having Antioxidant properties. In *Brassica alba Boiss* L., the presence of enzymes such as Superoxide dismutase, Glutathione peroxidase[36] plays a biological role in protecting the organism from oxidative damage. Piperine[27] of *Piper longum* L., Terpenoids[28] (α-pinene) of *Piper nigrum* L., Polyphenols, β carotene[29] of *Zingiber officinale* Roscoe., α-Terpinol acetate[33] of *Elettaria cardamomum* L., β-Pinene, Limonene[37] of *Ferula assafoetida* L. and Alliin[39] of *Allium sativum* L. are some other examples for chemical constituents with Antioxidant activity.

Parmacological actions like Anti-asthmatic, Anti-obesity, Anti-diabetic, and Blood purifying can also be seen in drugs mentioned in the *Katukaskandhaya.*

By comparing and analyzing the pharmacological, therapeutic and chemical properties of the selected drugs, it was clear that the drugs mentioned in the *Katukaskandhaya* had many similar features in all three aspects. This gave a comprehensive analysis with reference to facts mentioned in Ayurveda, the Traditional system of medicine, Allopathic medicine and modern scientific knowledge.

Conclusion

Drugs with a pungent taste (*Katu rasa*) are critically important in balancing excess *Kapha dosha* as it is present with light, rough, sharp properties which eliminate the stagnant, heavy qualities of *Kapha dosha* from the body. These properties help to clear *Ama* (natural toxins) by stimulating the *Agni* (Digestive fire). Hot potency of these drugs also helps in pacifying both the *Kapha* and *Vata dosha*. Once the body is cleansed by removing the unwanted stagnation of vitiated *Dosha, Dhathu, Mala* and *Ama,* healthiness can be re-established and maintained. Antioxidant, Antimicrobial, Anti-inflammatory, Anti-carcinogenic, Neuroprotective, Cardioprotective, Respiratory protective, Anti-obesity, Anti-diabetic, and anti-hypertensive activities are some of the medicinal uses of the drugs of the *Katukaskandhaya.*

Results of the comprehensive analysis conducted with the ten ingredients can be utilized in gaining an overall idea about the medicinal importance and the disease conditions that can be treated by using the most appropriate drugs mentioned in the *Katukaskandhaya*.

Due to a lack of information regarding all the drugs mentioned under the group of Pungent taste

(*Katukaskandhaya*), the study was limited to the analysis of ten ingredients and in future this study will have proceeded with Phytochemical analysis and evaluation of therapeutic effect and efficacy of the compound drug formulae including the drugs of *Katukaskandhaya* in the management of *Kapha-Vataja* disorders.

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Value addition to rice bran oil as toilet soap

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Abstract

The oil extracted from rice bran is underutilized in Sri Lanka. It is a rich source of essential fatty acid linoleic acid. Linoleic acid is most favored in the production of cosmetic products for its emollient and moisturizing properties. The aims of this study were to extract rice bran oil from red, mixed and parboiled rice bran, prepare a composite crude rice bran oil sample, analyze crude composite oil for physicochemical characteristics stated in Sri Lanka Standard 1592: 2018, formulate rice bran oil enriched toilet soap and evaluate its compliance with the specifications mentioned in Sri Lanka Standard 34: 2009. Rice bran oil was extracted using hexane and composite crude rice bran oil was analyzed by methods mentioned in Sri Lanka Standard 313. Three trials were carried out for the formulation of a toilet soap by hot saponification reaction and methods in the International Organization for Standardization were followed to determine the characteristic requirements for the toilet soap. The highest oil yield (8.0%) was given by parboiled rice bran and composite rice bran oil showed 0.911 relative density, 1.456 refractive index, 103.122 g/100g of oil iodine value, 196.626 saponification value, 0.08% moisture and volatile matter, 16.0% free fatty acids and 30.0 meq/ Kg peroxide value. Trial 3 formulated soap showed 78.23 % total fatty matter, 1.8% matter insoluble in ethanol, 0.05% free caustic alkali and 0.2% total free alkali therefore complying with the requirements for a toilet soap.

Keywords: Rice bran oil, toilet soap, linoleic acid, total fatty matter

Introduction

Rice bran, a by-product of rice (*Oryza sativa*) milling is underutilized in Sri Lanka and is generally utilized as an animal feed. Rice bran is the cuticle between paddy husk and whole rice grain and contains 12-18% of oil which can be extracted by physical or chemical methods to acquire the value-added product rice bran oil (RBO) or rice oil [1]. RBO is renowned for its balanced fatty acid composition, and high levels of bioactive compounds such as γ-oryzanol, phytosterols, tocopherols, tocotrienols, squalene and several other nutrients. Due to its distinctive characteristics, RBO can be used for a variety of applications in both edible and non-edible forms. Lipase enzyme naturally present in rice bran is capable of hydrolyzing glycerides to free fatty acids and these free fatty acids together with phospholipids, glycolipids and waxes make crude RBO not suitable for direct consumption. However, free fatty acid-containing non-edible RBO can be incorporated into cosmetics, soaps, paints and detergents.

RBO is utilized as a raw material in personal care products such as soaps, shampoos and lubricants as a specialty ingredient for its cosmetic value. Essential fatty acid, linoleic acid (18:2) present in RBO in 28.0-53.4% content is capable of reducing trans-epidermal water loss thereby hydrating skin, preventing drying, scaling and cracking of the skin [2,3]. Linoleic acid shows emollient and moisturizing properties thereby helping in the healing process of dermatoses and sunburns and used for the treatment of *Acne vulgaricus.* In addition to linoleic acid, RBO is used as an ingredient in many cosmetics owing to

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the presence of a variety of antioxidants.

Soap enrichment with RBO helps with retaining healthy skin while eliminating a variety of skin conditions. Therefore, the incorporation of crude RBO for soap production is a value-added way out for the underutilization of rice bran in Sri Lanka. The objectives of this study were to extract oil from three rice bran types in Sri Lanka, prepare a composite crude rice bran oil sample, analyze extracted composite crude RBO for physicochemical characteristics stated in Sri Lanka Standard (SLS) 1592: 2018, formulate a toilet soap enriched with RBO and evaluate its compliance with specifications mentioned for toilet soap in SLS 34: 2009.

Materials and methods

Rice bran collection

Parboiled rice bran, red rice bran and mixed (red and white) rice bran were collected from rice mills at Polonnaruwa, Rathnapura and Gampaha respectively though there are some factors such as soil, climatic technological borne differences limiting the oil yield comparison.

Rice bran oil extraction

About 200 g of bran sample was refluxed with 400 mL hexane at 65 ºC for 3 hours in a round bottom flask. After cooling, the solution was filtered with a Whatman filter paper. Hexane was recovered by rotary evaporation. Extractions were carried out four times from each bran sample. Each extracted crude RBO sample was weighed and collected in an air-tight glass reagent bottle to produce the composite crude RBO sample (Figure 1).

Fig. 1. Composite crude RBO

Analysis of composite crude rice bran oil

Composite RBO was tested in triplicates for the following identity and quality requirements as described in SLS 1592: 2018 according to related procedures in SLS 313.

Relative density was determined according to SLS 313-1-2: 2009.

Refractive index was determined according to SLS 313-1-5: 2017.

Iodine value was determined according to SLS 313-2-2: 2019.

Saponification value was determined according to SLS 313-2-1: 2014.

Moisture and volatile matter were determined according to SLS 313-3-5: 2016.

Free fatty acids were determined according to SLS 313-2-6: 2009.

Peroxide value was determined according to SLS 313-3-7: 2017.

Development of value-added rice bran oil incorporated soap

Three trials happened to be carried out by hot process via saponification reaction using NaOH lye since the first and second trials failed to meet the required TFM for toilet soap. Constituent fats and oils for soaps in Trial 1 are; RBO 30% and coconut oil 70%, in Trial 2; RBO 30% and castor oil 70%, in Trial 3; RBO 30%, castor oil 55%, lanolin 10% and beeswax 5%. The saponification value for oil blends in each trial was determined according to SLS 313-2-1: 2014 procedure. Lye solutions for each trial were prepared by dissolving respective amounts of NaOH (obtained through saponification analysis) in distilled water. Each oil blend was heated in a beaker on a heating mantle. When the temperature reached to 60 ºC lye solution was added and mixed for 20 minutes at 100 rpm using an overhead stirrer. After the completion of the saponification, the resultant sodium salt of the fatty acids which is known as the soap is transferred into a mold and left at room temperature to solidify overnight prior to total fatty matter (TFM) determination.

Determination of total fatty matter for the developed three soap formulations

TFM was determined for all three trials according to the test method mentioned in ISO 685-1975 and the method was triplicated.

Rest of the parameters mentioned in SLS 34: 2009 were conducted on the trial which produced a TFM level above 76.5%.

Analysis of soap

The following parameters were determined for the soap with TFM above 76.5%. The methods were carried out in triplicates.

Matter insoluble in ethanol was determined according to ISO 673-1981 (E).

Free caustic alkali was determined according to ISO 456-1973 (E).

Total free alkali was determined according to ISO 684-1974 (E).

Results

Rice bran oil yield

The average crude RBO yield obtained for parboiled rice bran, red rice bran and mixed rice bran are 8.0%, 6.6% and 5.2% respectively.

Analysis of composite crude rice bran oil

Results obtained for identifying characteristics of extracted crude composite RBO sample are; relative index at 25 ºC 0.911 ± 0.000, the refractive index at 28.7 ºC 1.456 ± 0.003, iodine value 103.122 ± 1.616 g/100g of oil, saponification value 196.626 ± 1.819.

Composite crude RBO sample showed moisture and volatile matter at 103 ± 2 Cº 0.08 ± 0.00 %, free fatty acids as oleic acid 16.0 ± 0.2 % and peroxide value 30.0 ± 0.7 meq/ Kg as results for quality characteristics.

Analysis of soap

TFM of Trial 1, Trial 2 and Trail 3 soaps were 53.12%, 66.67% and 78.23% respectively.

Table 1 depicts the characteristic requirements mentioned in SLS 34:2009 for toilet soap and the mean results ± standard deviation (SD) obtained for the respective characteristics of Trial 3 soap.

Table 1. Results are shown in Trial 3

Characteristic	Requirement in SLS 34: 2009	Mean result ± SD
TFM, including rosin acids, percent by mass, min	76.5	78.23 ± 0.18
Matter insoluble in ethanol, percent by mass, max	2.0	1.8 ± 0.1
Free caustic alkali, as NaOH, percent by mass, max	0.06	0.05 ± 0.01
Total free alkali, as NaOH percent by mass, max	0.3	0.2 ± 0.1

Discussion

Rice bran is considered a low-oil source. The oil recovery from a low oil source is typically carried out by solvent extraction. In this study, hexane was used as the solvent for RBO extraction as it is the most common and conventional solvent for commercial RBO extraction owning to its low price and high extractability. Due to the fact that rice variety, growth climatic conditions and milling processes the extractable oil yield of different rice brans are obviously varied. Albeit the results of the oil yield obtained for different brans revealed that parboiled rice bran gives a higher percentage of oil compared to raw bran (red and mixed). This is due to the phenomenon of outward migration[4]. Parboiling results in the release of oil in rice grains which facilitates the effective extractability of oil.

According to SLS 1592: 2018 the relative index, refractive index, iodine value and saponification value requirements for RBO must be 0.910-0.929, 1.460-1.473, 90-115 and 180-199 respectively. Results obtained for these characteristics were well within each identity range, confirming the extracted crude RBO's originality and authenticity. Similarly, if crude RBO is later used for edible purposes it must comply with the following quality requirements. The moisture and volatile matter at 103 ± 2 Cº should not exceed 0.5% and the result conforms to this requirement. The maximum free

fatty acid content of crude RBO should not exceed 10% whereas the study result surpasses this quality requirement for crude RBO. This high value could be attributed to the natural development of acidity as a result of lipase activity during storage prior to extraction. The maximum peroxide value of the extracted crude RBO must be 20 meq/ Kg. However, the increased result in the study might be attributed to the formation of oxidative products (mainly peroxides) during storage.

Soap is used as a cleansing agent on a daily basis and TFM is a quality parameter that is used to grade soaps. The high content of TFM is an indication of high-quality soap. According to SLS, TFM above 76.5% is considered good quality while TFM below 40% is the lowest acceptable soap safe for use. High TFM soap is capable of rehydrating and smoothening skin while acting as a lubricant owning to its high oil content [5]. The presence of unreacted lye lowers the TFM value degrading soap quality, consequently drying the skin. From the results obtained, Trial 3 soap is showing the highest quality owning to its 78.23% TFM value. Trial 1 and Trial 2 soaps can be regarded as bathing bars since the minimum requirement for a bathing bar is 40% according to SLS 1220: 2016. Trial 3 soap analysis results obtained for matter insoluble in ethanol, free caustic alkali and total free alkali were within the standard range further confirming its compliance to the toilet soap standard SLS 34: 2009 and therefore accepted as a toilet soap.

Conclusion

Trial 3 soap complies with the requirements mentioned in SLS 34: 2009. Hence, Trial 3 soap shall be regarded as toilet soap.

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