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LSP Kumaradharmasena
MD Scholar,
Department of Shalya Shalakya,
Institute of indigenous Medicine,
University of Colombo,
Rajagiriya, Sri Lanka.

LDAM Arawwawala
Senior Research Scientist,
Industrial Technology Institute,
Bauddhaloka Mawatha,
Colombo 7, Sri Lanka.

PIPK Fernando
Laboratory Technologist,
Industrial Technology Institute,
Bauddhaloka Mawatha,
Colombo 7, Sri Lanka.

KPP Peiris
Senior Lecturer (Gr.1), Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka.

SV Kamal
Senior Lecturer (Gr.1),
Department of Shalya Shalakya,
Institute of indigenous Medicine,
University of Colombo,
Rajagiriya, Sri Lanka.

Correspondence:
LSP Kumaradharmasena
MD Scholar,
Department of Shalya Shalakya,
Institute of indigenous Medicine,
University of Colombo,
Rajagiriya, Sri Lanka.

Quality assessment of *Mustadi Taila*: An Ayurvedic oil as a remedy for Dental Caries (*Krimi Danta)*

LSP Kumaradharmasena, LDAM Arawwawala, PIPK Fernando, KPP Peiris, SV Kamal

Abstract
The disease "*Krimi danta*" has been described under the caption of *Danta Roga* which is one of the diseases of *Mukha Roga*. If not treated properly this oral disease may be gradually results in tooth loss. As the modern point of view, the disease, "*Krimi danta*" is known as "Dental Caries". *Mustadi Taila*, is a medicated oil prescribed in *Chakra Datta* as a remedy for Dental Caries.
In the present study, an attempt was taken to assess the quality of *Mustadi Taila*. Organoleptic properties (colour, smell, appearance, taste) and physico - chemical parameters (specific gravity, saponification value, peroxide value, acid value and iodine value) were determined in order to assess the quality of the medicated oil. Thin Layer Chromatography (TLC) fingerprint was developed for dichloromethane fraction of the oil using methanol: cyclohexane and dichloromethane in a ratio of 0.3:2.0:7.7. Further, phytochemical screening, microbiological and heavy metals limits were determined in the oil using standard protocols.
According to the results, *Mustadi Taila* appeared to be brownish orange viscous oil with characteristic sesame oil odour and pleasant taste. In addition, specific gravity, saponification value, peroxide value, acid value and iodine value were 0.9225 ± 0.0003, 211.3±0.9 mg/g, 3.0±0.1milliequalents/kg, 3.8±0.1 mg KOH/g and 96.0±1.2 $I_2$100/g respectively. TLC fingerprint profile consists of 10 prominent spots under UV light. Phytochemical screening revealed the presence of alkaloids, steroids, tannins, saponins and flavonoids. Further, very few microbial counts were observed and heavy metals (Hg, As, Pb, Cd) were not detected in the oil. In conclusion, present study reveals the quality of *Mustadi Taila* for the first time.

Keywords: *Mustadi Taila*, Physico-chemical parameters, Quality

1. Introduction
Man has given importance to dental health and hygiene because of health, beauty, purity, of language and politeness. The healthy teeth are very much necessary for healthy body. According to the World Health Organization, "Oral health is essential to general health and quality of life. It is a state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial wellbeing" [1].
Oral health problems arise mainly as a result of two oral diseases such as Dental Caries and Periodontal Diseases. Although, the prevalence of these two diseases are changing, still it remains true that virtually every adult in the world has experience of either Dental Caries or Periodontal Diseases or both [2]. More than 50% of Sri Lankan population and 36% of world population are suffering from Dental Caries [3]. School children ranging from 60% - 90% in the world and nearly 100% of adults have dental cavities that is leading to pain and discomfort [4]. Therefore, Dental Caries still remains a global problem. There is presently an alarming rate of increase in the prevalence of Dental Caries in developing countries. The introduction of sucrose into the modern diet has been associated with the increased caries prevalence. Dental caries is the most prevalent chronic disease affecting the human race. Once it occurs, its manifestations persist throughout life even though the lesion is treated. There are practically no geographic areas in the world whose inhabitants do not exhibit some evidence of Dental Caries it affects persons of sexes and all races, all socio-economic strata and every age group.
The disease "*Krimi danta*" has been described under the caption of *Danta Roga* which is one of the diseases of *Mukha Roga*. As the modern point of view, the disease, "*Krimi danta*" is known as "Dental Caries". Dental Caries is a multi-factorial disease requiring the presence of a susceptible host, cariogenic micro flora, time and a diet conducive to enamel demineralization.

It is a disease that dates back to antiquity and occurs in populations that have never used sugar or processed foods [5].
Considering all the above points, it has been thought to develop an effective therapy, which have been claimed to possess a definite curative effect and also as a preventive remedy on this world wide problem. Ayurveda is more than 3000 years old adopting healing traditional method in ancient Indian culture. The ancient Indian physicians wrote authentic texts using their own experience. The treatment of dental diseases is one of the oldest forms of medico-surgical handicraft. *Mustadi Taila*, is one of the medicated oils consists of 8 medicinal plants (Table 1) prescribed in *Chakra Datta* [6] as a remedy for Dental Caries. In the present investigation, an attempt was made to assess the quality of the *Mustadi Taila* using standard protocols.

Table 1: Plant ingredients of Mustadi Taila

Plant ingredients	Parts of the plant
Cyperus rotundus L.	Rhizome
Rubia cordifolia L.	Root
Glycyrrhiza glabra L.	Root
Vitex negundo L.	Root
Cedrus deodara Roxb.	Stem
Acacia chundra Willd.	Stem
Vetiveria zizanioides L.	Root
Embelia ribes Burm. F.	Seeds

2. Material and Methods

Pharmacognostically pure and authentic ingredients were used for the preparation of *Mustadi Taila*. The herbarium sheets and raw materials were authenticated (specimen no: RM 1-8) by the Senior Scientist, Botany Section, Bandaranayaka Memorial Ayurveda Research Institute, Nawinna, Maharagama, Sri Lanka.

2.1. Preparation of *Mustadi Taila*

Mustadi Taila was prepared at Pharmacy, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka according to the method described in Sharangadara Samhita. In brief, all the ingredients were washed thoroughly and air dried. Then they were separately pulverized to a coarse powder and each ingredient was added to a stainless steel vessel. After that, *Kwata* was prepared by adding water into the stainless steel vessel containing all the plant ingredients and the mixture was heated using mild flame until the volume of the water reduced to one fourth of the original volume. The *Kwata* was filtered through a muslin cloth. *Murchita Tila Taila* was added to a copper vessel and mixed the *Kwata* while stirring the mixture, exposed to the heat to evaporate the moisture. The *varti* was exposed to the flame and confirmed the absence of cracking sound indicating absence of moisture. Finally, oil was filtered through a muslin cloth and allowed to cool. The samples were stored in air tight sterilized containers until use.

2.2. Evaluation of organoleptic properties and physico-chemical parameters

Organoleptic properties **(**colour, smell, appearance) and physico-chemical parameters (specific gravity, saponification value, peroxide value, acid value, iodine value) were determined in *Mustadi Taila* according to the standard protocols.

2.2.1. Determination of specific gravity

Firstly, empty specific gravity bottle was weighed and then filled with distilled water and weighed. After that, specific gravity bottle was well dried and filled with *Mustadi Taila* and weighed. The difference in weights (weight of the specific gravity bottle filled with oil - weight of the empty specific gravity bottle) was divided by the weight of an equal volume of water to give the specific gravity of *Mustadi Taila.*

2.2.2. Determination of saponification value

In brief, oil sample was accurately weighed (about 1.5g) and added into a conical flask. Then alcoholic potassium hydroxide (25 ml) was added and connected the reflux air condenser to the flask. The flask was heated on the water bath for 1h. When the flask and the condenser were cooled, inner wall of the condenser was washed with 10 ml of ethyl alcohol. Finally, 1 ml of phenolphthalein was added and titrated with standard hydrochloric acid. A blank titration was done at the same time [7].
Calculation:

$$\text{Saponification value} = \frac{56.1\ (B - S)\ N}{W}$$

B= Volume in ml of standard hydrochloric acid required for the blank
S = Volume in ml of standard hydrochloric acid required for the sample
N= Normality of the standard hydrochloric acid
W = Weight in g of the material taken for the test

2.2.3. Determination of peroxide value

In brief, oil sample was accurately weighed (5 g) into a glass stopper conical flask. Acetic acid – chloroform solution was added into the conical flask and swirled the flask until the sample was dissolved. Then 1 ml of saturated potassium iodide was added, allowed the solution to stand exactly one minute with occasional shaking and then added 75 ml of distilled water. Finally, titrated with 0.1 N sodium thiosulphate solution with constant and vigorous shaking. Continued the titration until the yellow colour almost disappears and added 0.5 ml of starch solution and continue titration till the blue colour just disappears [8]
Calculation:

$$\text{Peroxide Value} = \frac{(S - B) \times N \times 1000}{W}$$

Where,
S = Volume in ml of sodium thiosulphate solution required for the sample
B = Volume in ml of the sodium thiosulphate solution required for the blank
N = Normality of the sodium thiosulphate solution
W = Weight in g of the material taken for the test

2.2.4. Determination of acid value

In brief, oil sample was accurately weighed and added into a 200 ml conical flask. Then 100 ml of freshly neutralized hot ethyl alcohol and 1 ml of phenolphthalein were added to the

above mixture, boiled for 5 minutes and titrated as hot as possible with standard 0.25 N KOH solution, shaking vigorously during titration[8].
Calculation:

$$\text{Acid value} = \frac{56.1 \times V \times N}{W}$$

Where
V = Volume in ml of standard KOH solution required for the sample
N = Normality of standard potassium hydroxide solution
W = Weight in g of the material taken for the test

2.2.5. Determination of iodine value
Oil sample was accurately weighed (5 g) into the iodine flask, 25 ml of carbon tetrachloride was added and dissolved the contents. Then, 25 ml of the Wijis solution was added, replaced the glass stopper after wetting with potassium iodine solution and allowed to stand in dark for 1 h. A blank test was carried out under similar experimental conditions. Then, potassium iodine solution (15 ml) and water (100 ml) were added. Finally, liberated iodine was titrated with standard sodium thiosulphate solution. Swirled the contents of the bottle continuously, until color of the solution was straw color. Then added the starch solution (1 ml) and continued the titration until the blue color disappeared[7].
Calculation:

$$\text{Iodine Value} = \frac{12.69\ (B - S) \times N}{W}$$

Where,
B = Volume in ml of standard sodium thiosulphate solution required for the blank
S = Volume in ml of standard sodium thiosulphate solution required for the sample
N = Normality of the sodium thiosulphate solution
W = Weight in g of the material taken for the test

2.2.6. Development of Thin Layer Chromatography (TLC) fingerprint
Oil (50 ml) was added to a round bottom containing 100 ml of water and refluxed for 1 h. Then, water layer was separated and added to a separating funnel containing hexane. After that, hexane fraction was removed and dichloromethane (25 ml) was added and shaken well. Finally, dichloromethane layer was separated, concentrated and spotted on a TLC plate. Thin Layer Chromatography (TLC) fingerprint was developed for dichloromethane fraction of the oil using methanol: cyclohexane and dichloromethane in a ratio of: 0.3:2.0:7.7.

2.2.7. Phytochemical screening
Presence or absences of phytochemicals such as alkaloids, saponins, steroids, tannins and flavonoids were determined according to the standard protocols [9].

2.2.8. Microbiological limits
Limits of Aerobic plate count, *Staphylococcus aureus*, Coliforms, *Escherichia coli* and Yeast and Moulds were determined in *Mustadi Taila* according to the methods described in SLS standards [10-13].

2.2.9. Heavy metal analysis
Quantitative determination of Arsenic [14], Mercury [15], Cadmium [14] and Lead [14] were carried out in *Mustadi Taila* according to relevant methods described in AOAC methods.

3. Results and Discussion
The physico-chemical parameters of *Mustadi Taila* are shown in Table 2. Normally, oils give different characteristic color and odour relative to the herbs and other materials which were used to prepare the oil.

Table 2: Organoleptic properties and physico-chemical parameters of *Mustadi Taila*

Parameters	***Mustadi Taila***
Colour	Brownish orange
Smell	Characteristic Sesame oil odour
Appearance	Viscous
Taste	Pleasant taste
Specific Gravity	0.9225 ± 0.0003
Saponification Value	211.3±0.9 mg/g
Peroxide Value	3.0±0.1milliequalents/kg
Acid Value	3.8±0.1 mg KOH/g
Iodine Value	96.0± 1.2 $I_2$100/g

Saponification value is the number of milligram of potassium hydroxide required for neutralizing the fatty acids [16]. It is a measure of the average molecular weight of all the fatty acid present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids [17]. In general, saponification value of plant origin oils ranging from 188- 196 mg/g [18]. However, in the present study, slight deviation was observed with saponification value (211.3±0.9 mg/g) of *Mustadi Taila.*
The acid value is the mass of potassium hydroxide in milligrams that is required to neutralize one gram of chemical substance. Acid value is used to quantify the amount of acid present in an oil sample [19]. According to the results, acid value of *Mustadi Taila* was 3.8±0.1 mg KOH/g. similar acid values were observed for other medicated oils [20] also. The peroxide value is a measure of the active oxygen in the oil and the potential to go rancid. High starting levels of peroxide values are a bad sign.
According to the Sri Lanka Standards[8], upper limit of peroxide value is 10 milliequivalents/kg for fixed oils. Peroxide value of the *Mustadi Taila* was 3.0±0.1milliequalents/kg. In general, peroxide levels lesser 10 milliequivalents/kg means that oil is stable with a longer shelf life.
The iodine value is a measure of the degree of unsaturation in oil and could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation [21]. According to the present study, iodine value of *Mustadi Taila* was 96.0± 1.2 $I_2$100/g. TLC fingerprint profile consists of 10 prominent spots under UV light (at both 254 nm and 366 nm). Qualitative tests are used to detect the presence of some major phytochemicals which play a very important

role in the expression of biological activities. Phyto-chemical screening revealed the presence of alkaloids, steroids, tannins, saponins and flavonoids in the oil. Moreover, very few microbial counts (Table 3) were observed and heavy metals (Hg, As, Pb, Cd) were not detected in the oil.

Table 3: Microbial Counts of *Mustadi Taila*

	Microbial Counts
Aerobic plate count/ml	Less than 1
Yeast and mould/ml	1.0×10^1
Coliforms (MPN)/ml	Not detected
Escherichia coli (MPN)/ml	Not detected
Staphylococcus aureus/ml	Less than 10

4. Conclusion

The quality control parameters resulted after scientific evaluation of *Mustadi Taila* can be used as reference standard for quality control protocols in order to have a proper quality check over its preparation and processing. In conclusion, present study reveals the quality of *Mustadi Taila* for the first time.

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