Asian Journal of Pharmacognosy

Research Paper

Quality assessment of *Bilvadi taila* used in Sensorineural deafness

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(Accepted January 3, 2024)

***Abstract**:* An attempt was taken to assess the quality of *Bilvadi Taila* which is composed of dried unripe fruit of *Aegle marmelos* L, seed oil of *Brassica nigra* L, goat milk, cow urine and water. Quality control parameters such as physico-chemical, microbial counts, and heavy metals were determined. In addition, phytochemical screening and development of Thin Layer Chromatography (TLC) fingerprint for the *Bilvadi* Taila were carried out. Results revealed that the peroxide value, acid value, iodine value, saponification value, moisture content, and reflective index of the *Bilvadi Taila* were 45.5±0.5 milliequivalents per kg, 22.0±0.4 mg KOH/g, 1.468±0.200 (at 40 °C), 190.4±1.2mg KOH/g, 59.0±1.6%w/w and 0.4±0.0%w/w respectively. Micro-organisms (Mesophilic bacteria, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Candida albicans*) and heavy metals (Hg, As, Pd, and Cd) were not detected in the *Bilvadi Taila*. Seven and ten spots were observed in the Thin Layer Chromatography fingerprint of the *Bilvadi Taila* before and after spraying vanillin sulphate respectively. Alkaloids, steroids, tannins, saponins, and flavonoids were found to be present in the *Bilvadi Taila*. In conclusion, an attempt was made to establish the quality control parameters of *Bilvadi Taila* for the first time.

***Keywords**: Bilvadi Taila*, Physicochemical parameters, Phytochemicals, Sensor neural deafness

eISSN-0128-1119

INTRODUCTION

Hearing is an essential sense of the individual, which is crucial for verbal communication and social well-being. There are different types of hearing loss; Conductive hearing loss, Sensorineural hearing loss, and mixed types (Martinez & Yetman, 2020). Over 5% of the world population (432 million adults and 34 million children) require rehabilitation to address their "disabling" hearing loss. It is estimated that by in the year 2050 over 700 million people or one in every ten people will have disabling hearing loss. Because, with increasing noise pollution, and usage of mobile phones, the incidence is gradually increased. Reported data confirmed that 1.1 billion young people aged between 12-35 years are at risk of hearing loss. Disabling hearing loss refers to hearing loss greater than 35 decibels (dB) in the better-hearing ear. The prevalence of hearing loss increases with age, among those older than 60 years; over 25% are affected by disabling hearing loss (World Health Organization, 2021). Sensorineural deafness occurs when there is a problem in the sensory apparatus such as the cochlea, organ of coti or in the pathways of conduction of nerve impulses to the auditory cortex of the brain. Sensor neural deafness can be congenital or acquired. Congenital sensor neural hearing loss is present at birth, due to genetic causes, causes affected during pregnancy or causes during delivery of the baby. Not only that but also several acquired causes responsible for this type of deafness.

In Ayurveda, this Sensorineural deafness can be correlated with *Karna Badhirya* (Susrutha nidana) due to its signs and symptoms, especially poor speech discrimination. *Badhirya* is one of the most common audiological disorders and the commonest challenging problem of all Ear, Nose and Throat (ENT) surgeons. The disease looks simple but it doesn't bring satisfactory relief to the patients. Thus, the treatment adopted for *Badhirya* in the modern system of medicine has not yet been satisfactory. Cochlea Implant (Surgical Intervention) is rare in these cases performed only when presented with complications. Besides these, Ayurveda also offers different kinds of treatments in the management of *Karna Badhirya*. Among them, *Karnapoorana* is one of the most effective local treatment procedures indicated in the management of sensorineural deafness (Susrutha nidana). When the eardrum is in an intact position, gently filling the ear canal with suitable medicated oil or decoction and keeping for a specific time period is known as *Karna poorana*. Therefore, in Shalakya clinic of Gampaha Wickramarachchi Ayurveda Teaching Hospital has been used *Bilvadi taila* since long period for *karnapoorana* as an effective local treatment for *Karna Badhirya* and its clinical effectiveness was confirmed. *Bilvadi Taila* is composed of dried unripe fruit of *Aegle marmelos* L. (Family: Rutaceae), seed oil of *Brassica nigra* L. (Family: Brassicaceae), goat milk, cow urine, and water. However, the quality control assessment of *Bilvadi Taila* has not carried out yet. Therefore, an attempt was made to assess the quality of *Bilvadi Taila* using standard protocols.

MATERIALS & METHODS

*Preparation of oil: a*ll the ingredients of *Bilvadi Taila* were identified and authenticated by the Department of *Dravya Guna*, Faculty of Indigenous Medicine, The Gampaha Wickramarachchi University of Indigenous Medicine, Sri Lanka.

eISSN-0128-1119

Bilvadi Taila was prepared according to the *Sharangadhara Samhita Sneha paribhasha* (Sharangadhara, 1962).

Chemical testing of Bilvadi Taila: peroxide value and acid value (SLS 313 Part 3/Section 7:2009), iodine value (SLS 313 part 2/ Section 2:2014), and saponification value (SLS 313 part 2/ Section 1:2014) were determined according to the standard protocols.

Physical testing of Bilvadi Taila: Refractive index at 40°C (SLS 313 part 1/Section 5:2009) and moisture (SLS 313 part 3/Section 5:2009) were also determined using standard protocols.

Determination of microbial limits and heavy metals: presence or absence of microbial counts (Mesophilic bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*) and selected heavy metals (Hg, As, Pd and Cd) which are known to be high toxic to human being were determined using standard protocols (Kumaradharmasena et al., 2015).

Development of Thin Layer Chromatography (TLC) fingerprint profile: the oil was extracted into water and an aqueous layer was added to a separatory funnel containing 100 ml of dichloromethane mixed well and kept for 15 min. The dichloromethane fraction was collected without disturbing the aqueous layer. The same procedure was carried out for triplicates, pooled the dichloromethane fractions, and concentrated up to 10 ml. Finally, 10 µl of the dichloromethane fraction was spotted on the TLC plate and developed the TLC fingerprint using the following solvent system: Ethyl acetate: Dichloromethane: Cyclohexane (0.5:4.5:0.5 v/v). Observed the spots under UV lamp (at 254 nm and 366 nm) and calculated the R_f values of each spot. Then sprayed Vanillin sulphate, was heated at 110 ºC for 5 min, and calculated the R_f values of each spot gave a colour.

Phytochemical screening: The oil was extracted into water and the aqueous layer was subjected to screening phytochemicals such as saponins, tannins, phenols, alkaloids, flavonoids, terpenoids, sesquiterpenes, and steroids as described by Karunakaran and co-workers (2017).

RESULTS & DISCUSSION

In the present study, a quality assessment of *Bilvadi Taila* was carried out using the physical and chemical parameters. In addition, microbial purity and heavy metal analysis were also determined in the oil. Quality assessment is very important in herbal medicines such as medicated oil, tablets, capsules, powders, etc to maintain batch-wise consistency. Similar quality assessments were carried out for *Mustadi Taila* (Kumaradharmasena et al., 2015), *Vipadikahara grita Taila* (Hewageeganage et al, 2013) and *Tulsi Taila* (Shah and Patil, 2019). The acid value of an oil is known as the number of milligrams of potassium hydroxide required to neutralize the free acids of 1 g of oil. However, peroxide and acid values are high in the *Bilvadi Taila*. This may be due to

eISSN-0128-1119

the addition of goat milk and cow urine. As far as *Bilvadi Taila* is an external application, high peroxide and acid values may not have much harmful effect. The moisture content of *Bilvadi Taila* is very low. The saponification value or number pertains to all fatty acids present in the sample (free and esterified). Both saponification and refractive index could be considered to detect the batch-wise consistency of the oil when formulated on a large scale (Table 1).

Table 1. Physico-chemical parameters of *Bilvadi Taila*

Test / Unit	Test Value
Peroxide value, milliequivalents per kg	45.5±0.5
Acid value, mg KOH/g	22.0±0.4
Refractive index at 40 °C	1.468±0.200
Saponification value, mg KOH/g	190.4±1.2
Iodine value, percent by mass	59.0±1.6
Moisture, percent by mass	0.4±0.0

Results expressed as Mean ±S.E.M; n=3

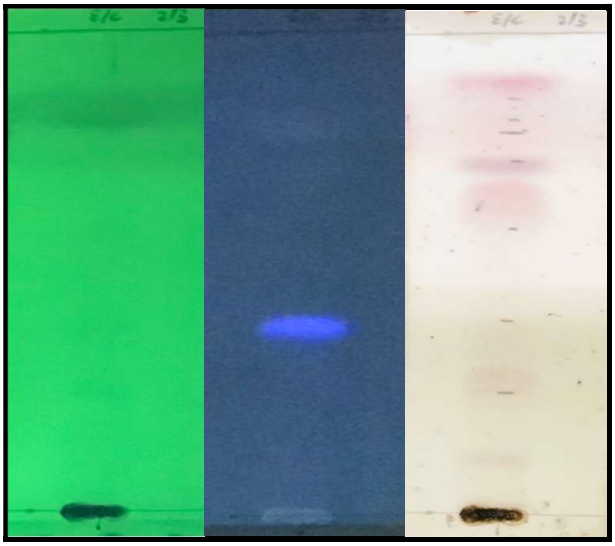
Moreover, no tested micro-organisms and heavy metals were found in *Bilvadi Taila* (Table 2). This is a good safety indicator of *Bilvadi Taila*.

Table 2. Microbial counts and heavy metals in *Bilvadi Taila*

Test / Unit	Test Results
Mesophilic bacteria, per g	Absent
Staphylococcus aureus, per 10 g	Absent
Pseudomonas aeruginosa, per 10 g	Absent
E. coli count, per 10 g	Absent
Candida albicans, per 10 g	Absent
Cadmium (as Cd), mg/kg	Not detected
Lead (as Pb), mg/kg	Not detected
Mercury (as Hg), mg/kg	Not detected
Arsenic (as As), mg/kg	Not detected

eISSN-0128-1119

Seven spots bearing the R_f values of 0.25, 0.41, 0.60, 0.74, 0.80, 0.83. 0.87 and ten spots bearing the R_f values of 0.12 (purple), 0.25 (purple), 0.29 (salmon pink), 0.34 (purple), 0.49 (purple), 0.56 (salmon pink), 0.72 (magenta), 0.79 (pink), 0.83 (pink), 0.90 (salmon pink) were observed in the Thin Layer Chromatography fingerprint of the *Bilvadi Taila* before (at 254 nm and 366 nm) and after spraying vanillin sulphate respectively (Figure 1). The phytochemicals such as tannins, phenols, flavonoids, alkaloids, saponins and flavonoids were found to be present in the *Bilvadi Taila* while steroids and terpenoids were absent (Table 3).



254 nm 366 nm after spraying

Figure 1. TLC fingerprint profiles of *Bilvadi Taila*

Table 3. Phytochemical screening of *Bilvadi Taila*

Type of phytochemical	Test	Result
Tannins	Ferric chloride test	++
	Lead acetate test	++
	Vanillin test	-
Phenols	Lead acetate test	++
	Vanillin test	-
Flavonoids	Dil ammonia+conc sulfuric acid	+
	Zn+conc HCl	-
Alkaloids	Wagner reagent	+
Terpenoids	Salkowski test	-
	Test for sesquiterpenes	-
Steroids	Acetic anhydride +Conc sulfuric acid	-
	Liberman Burchard test	-
Saponins	Froth test	+

+: present at moderate amount; ++: present at high amount; - negative

eISSN-0128-1119

CONCLUSION

An attempt was made to establish the quality control parameters of *Bilvadi Taila* for the first time. In future these data can be utilized as quality assurance reference standard for the *taila.* In addition; data can be utilized for Ayurveda pharmacopeia development. Despite centuries of searching, scientists and researchers have been unsuccessful in finding Sanjeevani. In August 2016, the state of Uttarakhand in northern India allocated 25 million rupees for a search expedition in the Dronagiri hill range near the Chinese border, where the herb is believed to exist. A recent development involves the research unit of the Uttarakhand Forest Department claiming to have discovered a herb in the Dronagiri hills that bears similarities to the mythical Sanjeevani plant mentioned in the Ramayana. However, further scientific study, including clinical trials and confirmation, is necessary before any definitive conclusions can be drawn.

DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

DECLARATION OF HONOUR

We declare on our honour that our results are not fake and made up.

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eISSN-0128-1119