PREPARATION AND STANDARIZATION OF ASANA BILVADI TAILA: CLASSICAL MEDICATED HAIR OIL

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ABSTRACT

Asana Bilavdi Taila is a classical combination of simple and routine herbs used in managing headache, migraine, and various types of eye diseases. In the present study, Asana bilavdi taila was formulated in laboratory by the formula given in an official ayurvedic treatise Sahasrayoga. The prepared oil was subjected to standardization. Standardization of herbal formulation is essential in order to assess the quality of drug for desired therapeutic value. By following the testing protocol of ASU drugs (Ayurveda, Siddha and Unani) various testing parameters including organoleptic and physicochemical tests for medicated oil like: acid value, sap value, rancidity, TLC, HPTLC etc. had been performed to fix the quality standards of the drug. This study results in a set of diagnostic characters, essential for its standardization. The values obtained after physicochemical study may be useful further to develop a new pharmacopoeial standards for the specific formulation. The parameters were found to be sufficient to standardize the Asana Bilvadi Taila.

KEY WORDS : Asana vilavadi taila, preparation, standardization, HPTLC, AAS.

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INTRODUCTION

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Traditional health care had been flourishing in this country for many centuries. Ayurveda and other Indian systems of medicine may be explored with the modern scientific approaches for better leads in the health care. In the last few decades, there is an exponential growth in the field of ayurvedic medicine [1]. Asana Bilvadi taila is made up of Tilataila (Sessamum indicum), Asana (*Pterocarpus marsupium*; Fabaceae), Bilva (*Aegle marmelos*; Rutaceae family) Yasthimadhu (*Glycyrrhiza glabra*; Fabaceae, Triphala (Amlaki, [*Emblica officinalis*; Phyllanthaceae], Haritaki [*Terminalia chebula*; Combrataceae], Behera (Terminalia bellerica; Combrataceae]). Bala (Sidacordifolia; Malvaceae), Sunthi (*Zingiber officinale*; Zingibereceae) khsira (cow's milk) as mentioned in Sahasrayoga [1-2]. It is useful in

headache, migraine and different types of eye diseases, ear diseases and cranium diseases [3]. It is also indicated in the rheumatic of head. There is great need of standardization and quality control of Ayurvedic formulations. The rising popularity of herbal products, both as food and food supplements and as phyto-therapeutic drugs has also given rise to many products describing adverse health effects and variable quality, efficacy and contents of herbal products. Side effects of herbal products may consist of allergic reactions and interactions with conventional drugs or intrinsic toxicity. These side effects are due to failure of Good Manufacturing Practice (GMP) in preparation which includes misidentification of plants, lack of standardization, contamination, substitution and adulteration of plants and incorrect preparations and/or dosages. This has necessitated the urgency of Ayurvedic Formulations' standardization. This work is undertaken with the objective of preparation and standardization of classical Ayurvedic hair oil- Asana Bilvadi Taila [4-5].

All the ingredients of the Asana Bilvadi Taila as Sessamum indicum, *Pterocarpus marsupium*, *Aegle marmelos*, *Glycyrrhiza glabra*, Triphala (*Emblica officinalis*, *Terminalia chebula*, Terminalia bellerica), Sidacordifolia, *Zingiber officinale*, Tinospora cordifolia and khsira had been recommend for headache (sirasula) since ancient times. It had been found to be used in the Chakshuroga(eye diseases), Karnaroga (Ear diseases) as mentioned in the classical texts [6].

EXPERIMENTAL SECTION

Materials and Methods

Plant materials

Asana Bivadi Taila consists of TilaTaila (Sessamum indicum), Asana (*Pterocarpus marsupium*; Fabaceae), Bilva (*Aegle marmelos*; Rutaceae family), Yasthimadhu (*Glycyrrhiza glabra*; Fabaceae), Triphala [Amlaki (*Emblica officinalis*; Euphorbiaceae), Haritaki (*Terminalia chebula*; Combretaceae), Behera (Terminalia bellerica; Combretaceae)] .Guduchi (Tinospora cordifolia; Menispermaceae) Bala (Sidacordifolia; Malvaceae), Sunthi (*Zingiber officinale*; Zingibereceae) khsira (Cow's milk). All the ingredients were collected during August 2013 from South 24 parganas, West Bengal, India. The plant materials were taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. Then the plant materials were maintained in our research laboratory for future reference. The plant materials were shade dried at room temperature with occasional shifting and then powdered with mechanical grinder, passing through sieve no. # 40 and was stored in an air-tight container.

Preparation of Asana Bivaditaila

In house formulation of Asana Bilvadi taila was prepared as per the ayurvedic treatise Sahasrayoga [1-2] as mentioned in Table 1.

Table 1: Composition of the Preparation [1]

Name of the ingredients	Botanical name	Parts used	Amount
Asana	*Pterocarpus marsupium* Roxb.	Heart wood	181.7 gm
Bilva	*Aegle marmelos* Linn	Fruit	250 gm
Guduchi	*Tinospora cordifolia* Wild.	Stem	225.5 gm
Bala	*Sidacordifolia* Linn.	Root	250 gm
Yasthimadhu	*Glycyrrhiza glabra* Linn.	Root	12 gm

Table 1: Composition of the Preparation [1]

Name of the ingredients	Botanical name	Parts used	Amount
Sunthi	*Zingiber officinale*	Rhizome	15 gm
Triphalachurna	*Emblica officinalis*Linn.	Fruits of Amlaki,	36.5 gm
	*Terminalia chebula*Linn.	Haritaki and Behera	
	*Terminalia bellerica*Roxb.		
Tilataila	*Sessamum indicum*Linn.	Seeds	600 ml
Cow milk			185 ml

Method of Preparation of the formulation [1]

Step I - At first take all the drugs taken were properly cleaned and then Asana, Bilva, Guduchi, Balamula were crushed up to Yavakutachurna (Coarse powder form). Then 4 times of water was added and boiled to reduce 1/4th, and then it was cooled and filtered and the filtrate was taken as decoction.

Step II - Yasthi, sunthi and triphala were made into paste with addition of very small quantity of water.

Step III - Oil was subjected to heat until the frothing stopped and smoke was appeared.

Step IV - After appearing of smokes the oil was removed from fire and the decoction, khsira and paste were added to the taila. Then heat was applied and paka (cooking) was continued until the water portion was evaporated completely.

Step V - After the evaporation of water portion medicated oil was screened through a clean cotton cloth at hot condition and the sediment apart was left. Then it was permitted to be self cooled and preserved it in a well closed container.

Physicochemical evaluations [7-9]

Organoleptic evaluation

Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc. The finished product was subjected for organoleptic test and the data were observed carefully.

Phytochemical tests

Preliminary qualitative phytochemical screenings were performed by application of various reagents to detect the presence of phytomolecules like- alkaloids, saponin, steroids, and fixed oil, phenolic compound, glycoside and carbohydrate etc. in the formulation. The data obtained were documented.

Physicochemical analysis

Physicochemical tests are necessary for oil preparation were performed; those are specific gravity, rancidity test, viscosity, refractive index, saponification value, peroxide value, iodine value, acid value, pH test etc. Under the instrumental analysis HPTLC, atomic absorption spectroscopy (AAS) was performed.

Microbial assay [10,11]

In microbiological assay- total microbial count and total aerobic count were performed under the standard aseptic conditions. Tests for specific pathogens like Escherichia Coli, Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus aureus were performed and the obtained results were compared to the standard value.

RESULTS AND DISCUSSIONS :

After performing the standardization of Asana Bilvadi taila the following results were found.

Oraganoleptic Evaluation

After performing the organoleptic tests, it was found that the oil was yellow in colour with characteristic odor of sesame oil. The results were summarized under Table 2.

Table 2 : Results of organoleptic evaluation

Parameters	Observation
Colour	Yellow colour
Odour	Vegetative odour /characteristic odour of sesame oil
Taste	Bitter taste

Phytochemical screening

After performing the qualitative phytochemical screening it was found that the prepared medicated oil was having the presence of alkaloid, saponin, steroid, phenolic compounds, glycosides etc. The results were summarized under Table 3.

Table 3 : Results of phytochemical evaluation

Specification	Tests Performed	Observation	Inference
Alkaloid	a.Mayer'sReagent	a.Cream precipitate	(+)
	b.Hager's Reagent	b.Yellowcolour appeared	
Tannin	$FeCl_3$ test	No green or blue colour appeared	(-)
Protein	a.Biuret test	a.Violetcolour not appeared	(-)
	b.Ninhydrin test	b.Violetcolour not appeared	
Saponin	Froth test	Froth appeared	(+)
Terpenoid	a.Libermann-	a. Colour changes not found	(-)
	Burchard's test	b. Colour changes not found	
	b.Noller'sTest		
Steroid	Libermann-	Reddish brown ring was observed	(+)
	Burchard's test		
Fixed oil	Spot test	Paper became transparent	(+)

Table 3 : Results of phytochemical evaluation

Specification	Tests Performed	Observation	Inference
Phenolic compound	a.Ammonia test b.Shinoda test	a.Yellow colour appeared on filter paper b.Magenta colour found	(+)
Glycoside	a.Legal's test b.Baljet's test	a.Pink or red colour appeared b.Orange colour appeared.	(+)
Carbohydrate	Molish test	Violet Purple colour appeared	(+)

(+)Present; (-)Absent

Physicochemical analysis

The data obtained after performing the physicochemical tests were summarized under Table 4.

Table 4 : Results of physicochemical tests

Parameters	Results obtained
Specific Gravity	0.9156
Rancidity test	No red color found
Viscosity	12.452 poise
Refractive Index	1.4733 at 25°C
Saponification Value	108.3
Peroxide Value	4
Iodine Value	100
Acid value	33.9966
pH	6

TLC technique: R_f value determination

Table 5: Solvent system - Benzene: Chloroform: Ethyl acetate = 4:3:3

R_f1	0.94828
R_f2	0.87931
R_f3	0.77586
R_f4	0.32759
R_f5	0.17241

Table 6: Solvent system - Benzene: Chloroform: Ethyl acetate = 5:3:2

R_f1	0.92857
R_f2	0.83929
R_f3	0.76786
R_f4	0.375

From the above result it was observed that the solvent system- Benzene: Chloroform: Ethyl acetate performed better separation than the other solvent system. So, in HPTLC this solvent system was applied. However no standard or marker was used.

Result of HPTLC :

HPTLC was only performed to obtain a clear chromatogram of the sample in a specific solvent system. HPTLC study also used to revel the most accurate R_f values.

Solvent system : Benzene: Chloroform: Ethyl Acetate = 3:4:3

Table 7: R_f values before derivatisation (at 366 nm)

R_f1	0.14
R_f2	0.40
R_f3	0.56
R_f4	0.63
R_f5	0.74

Table 8: R_f values after derivatisation with Anisaldehyde-sulphuric acid reagent (at 366 nm)

R_f1	0.20
R_f2	0.47
R_f3	0.76
R_f4	0.87

Atomic Absorption Spectrophotometry (AAS): The experimented results have been projected under Table 9.

Table 9: Results of Atomic Absorption Spectrophotometry

Sample name	Lead content	Arsenic content
Asana bilvadi taila	Below detectable limit **Limit of detection for Lead:** 0.08 p.p.m	0.05 p.p.m (passed the limit)

p.p.m. = Parts per million

Biological evaluation :

After performing the analysis, it was found that the medicated oil was free from microbial contamination; even it was passed the microbial limits for specific pathogens. The results were summarized under Table 10 and Table 11.

Table 10 : Results of Total Microbial count

TYPE OF STUDY	MEDIA USED	REPORT
Total aerobic count	Nutrient broth agar medium	Growth was not found
Total fungal count	Czapekdox agar medium	Growth was not found

Table 11: Results of Tests for the presence of Specific Pathogens

NAME OF PATHOGEN	MEDIA USED	REPORT
Escherichia. coli	EC broth agar medium	Growth was not found
Salmonella sp	Salmonella differential agar medium	Growth was not found
Pseudomonas aeruginosa	Cetramide agar medium	Growth was not found
Staphylococcus aureus	Mannitol salt agar medium	Growth was not found

Asana vilvadi taila is a unique combination of ayurvedic herbs having sirovirechana activity along with nourishment and health promoting action. Apart from the key ingredients tila taila, which is used as a base is itself acts as analgesic, anti-inflammatory and cooling effects, the khirapakakalpana (cooked with milk) adjoins the calm and cooling effects and imparts the Rasayana and Medhya (intellectual) property to the formulation.

After performing the organoleptic analysis, it was found that the oil having yellow colour and vegetative smell and bitter taste. Preliminary phytochemical studies revealed the presence of alkaloids, saponin, steroids, and fixed oil, phenolic compound, glycoside and carbohydrate in the formulation. After performing the physicochemical analysis, it was found that the Specific Gravity, Viscosity, Refractive Index, Saponification Value, Peroxide Value And Acid Value of Asana vilvadi taila were 0.9156, 12.452, 1.4733, 108.3, 4.0 and 33.9966 respectively; in comparison to tila taila (sesame oil) the specific Gravity, Saponification Value, Peroxide Value and Acid Value is 0.9160, 180.0, 6.0 and 35.66 respectively. Therefore, it can be concluded that the prepared oil had some variable data with original tila taila. Instrumental analysis such as detection of heavy metals with the help of Atomic Absorption Spectroscopy (AAS) detected no harmful quantity of heavy metals in the oil. Then biological analysis revealed that the oil did not contain any specific pathogen or any bacteria or fungi. Therefore, the process could be followed as a standard operating procedure for the processing of Asana vilvadi taila.

Therefore, the combination of all ingredients in one formulation would claim for its nourishing activity to the head as well as eyes and ears.

CONCLUSION

After performing the experiment, it can be concluded that all the physicochemical and instrumental data produced fruitful results which supported the standard quality of the

prepared oil. The data may be further used to build up a complete monograph of Asana vilvadi taila.

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