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Standardization of Tulsi Taila: An Ayurvedic oil based medicine.

Shruti Shah*, Sonali Patil

B. K. Birla College of Arts, Science and Commerce (Autonomous), Kalyan, MS, INDIA

ABSTRACT

Tulsi (*Ocimum sanctum*) is cultivated near Hindu houses and temples throughout India. In Ayurvedic medicine, leaves, seeds and root of this plant are being used either alone or in combination with others. The chemical composition of Tulsi is highly complex, containing many nutrients and other biological active compounds.Ayurvedic formulations are safe and effective but adulteration of lower cost material in it reduces the quality of the drug, hence the standardization of herbal drugs is necessary. Present study involves preparation and standardization of Tulsi oil for determination of its standard parameters as per monograph to maintain the safety, uniformity and quality production of the product. Essential oil of Tulsi extracted from holy basil (*Ocimum tenuiflorum*) which is then used in combination with coconut oil and fenugreek seeds.The major uses are as bug repellent,deodorizer, for releivijng skin problems, dental problems, in fever,chronic cough and cold, bronchitis etc. The parameters tested for the Tulsi oil include Organoleptic evaluation, physicochemical and Phytochemical evaluation,chromatographic analysis for active constituents and stability studies. All the parameters were tested as per pharmacopeia standards.

Keywords: standardization, tulsi oil, ayurveda., essential oil.

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***Address for Correspondence:**

Shruti Shah, B. K. Birla College of Arts, Science and Commerce (Autonomous), Kalyan, MS, INDIA

1. INTRODUCTION:

Ayurveda means the science of life. Charaka defines "That science is designated as Ayurveda which deals with advantage and disadvantage as well as happy and unhappy states of life along with what is good and bad for life, its measurement and the life itself (Charaka Sutra 1 - 4)".

Generally, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being safe and effective (EMEA, 2005; WHO, 2002c, 1998c, 1996, 1991a,b, 1990, 1988). The term "herbal drugs" denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage (EMEA, 1998). Hence they are capable of variation. This variability is also caused by differences in growth, geographical location, and time of harvesting. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards.Hence standardization is a tool in the quality control process.

Tulsi oil is extracted from holy basil (*Ocimum tenuiflorum*), a species of basil that is native to the Indian subcontinent. The tulsi plant is highly sensitive to frost and thrives best in warm Mediterranean climates, just as other basil species do. But while it grows best out in the garden, you can still grow this herb indoors. Because of its many beneficial properties, tulsi has been dubbed the "Queen of Herbs. The name itself translates to "the incomparable one," which probably refers to the numerous health benefits it can provide. Tulsi oil, in particular, is said to be effective for warding off insects, when used as a bug repellent.Diluted tulsi oil may also work as a good deodorizer to help eliminate body odor.It's helping relieve skin problems. The eugenol in tulsi may help manage cholesterol levels in the blood.It is an excellent germicidal, antibiotic, fungicidal and disinfectant, and may efficiently protect your body from pathogenic organisms.It helps relieve respiratory congestion.There are various types of tulsi oil from various manufacturers are available in market.For the current research,tulsi oil was prepared at laboratory level as per the standard literature and standardization of it was achieved.

2. MATERIALS AND METHODS

2.1 Collection, identification and authentication of raw materials

Tulasi plant collected from the local region, and plant Authenticated from the botany department. Coconut oil was procured from local ayurvedic store.

2.2 Method of preparation of Tulasi Taila

Tulsi leaves were washed , cut and ground until they form a paste. Three tablespoons of paste was mixed with 130 ml of coconut oil and put over low heat. 250 gm of fenugreek seeds were added.Once the seeds started to pop turn off the heat.

Table 1: Ingredients for Tulsi taila

SR. NO.	MATERIALS	QUANTITY
1.	Tulsi Leaves	3 Tablespoon
2.	Fenugreek Seeds	250 gm
3.	Coconut Oil	130 ml

2.3. Evaluation of Taila

2.3.1 Organoleptic evaluation

The organoleptic evaluation of prepared oil was performed for the color, odor, and texture

2.3.2 Phytochemical evaluation

Qualitative phytochemical evaluation of methanolic extracts of raw material and laboratory prepared Tulasi oil was performed according to the standardization parameters.Presence of phytoconstituents like tannins,glycosides,saponins,phenolic compounds,alkaloids,terpenoides,steroids,flavanoides was checked.

2.3.3 Physicochemical evaluation

The formulation was studied for physicochemical parameters like specific gravity,Peroxide value, Saponification value and acid value.

2.3.4 Chromatographic analysis

2.3.4.1 Thin Layer Chromatography was performed to analyze the presence of raw materials in formulation and to achieve fingerprint of the formulation. Methanolic extracts of raw materials and formulations were used as per cchromatographic conditions (table 2)

Table 2: Chromatographic Conditions for HPTLC

Mobile phase	Toluene : Ethyl acetate : Formic acid : Glacial acetic acid (5 : 5 : 1: 1)
Sample	Methanolic extracts of raw materials and formulation
Detection	UV light 254 nm

2.3.4.2. Detection of marker compound Eugenol in formulation

High Performance Liquid Chromatography was used for separation and identification of active constituent Eugenol in the formulation by comparing with standard Eugenol.The analysis was done using reverse phase chromatography as per chromatographic conditions (table 3)

Table 3: Chromatographic Conditions for HPLC

Column	C18
Mobile Phase	methanol- acetonitrile- water in volume ratio of 10 : 50 : 40
Flow rate	1ml/min
Detection	280 nm

3. RESULT AND DISCUSSION

Standardization was performed for laboratory prepared Tulasi oil. Tulasi plant collected from the local region of Kalyan and Authenticated from the Botany department of B.K.Birla College,Kalyan . Tulasi oil was prepared as per the standard ayurvedic literature and standardized according to standard parameters.

Organoleptic properties of Tulasi oil are found as per the standard requirement (table 4).

A phytochemical investigation revealed that Tulasi oil mainly contained Alkaloids, Glycosides,Tannins and Terpenoides (table 5).

Physicochemical analysis was done as per the protocols of ayurvedic pharmacopeia and parameters like specific gravity,peroxide value,saponification value and acid value were found as per the standard limits (table 6)

Overnight extraction using methanol was done for raw materials as well as formulation.The extracts were subjected to thin layer chromatography and it was found that bands achieved in raw materials were matching with that of formulation .Thus presence of raw material and phytoconstituents was confirmed.This can also be termed as TLC fingerprint of formulation (Fig 1)

From the formulation extract, eugenol was separated , identified and confirmed by using High Performance Liquid chromatography.The extracts were compared to standard eugenol. Retention time of eugenol in extract was found to be 6.7 minutes which was matching with that of standard(Fig 2).

Table 4: Organoleptic Test

Sr. No.	Parameters	Observation
1	Colour	Greenish Brown
2	Appearance	Oily
3	Texture	Liquid
4	Consistency	Smooth
5	Odour	Woody

Table 5: Phytochemical Test for Raw Material & Formulation

Sr. No.	Phytoconstituents Test	Observation	Result	
			Tulsi	Tulsi oil
1.	Tannins 0.5g dried sample +boil with 20 ml water+ drops of 0.1% $FeCl_3$	Dark colored ppt.	P	P
2.	Glycosides 5 ml aq. extract+ 2 ml glacial acetic acid with $FeCl_3$+ 1 ml H_2SO_4	Brown ring	P	P
3.	Terpenoids 5 ml aq. extract+ 2 ml chloform+ 3 ml conc. H_2SO_4	Yellow colour	A	A
4.	Alkaloids 1 ml aq. extract+ conc. HCl + Hager's reagent	Yellow ppt.	P	P
5.	Flavonoids 5 ml dil.NH_3+aq. extract + conc. H_2SO_4	Yellow colour disappears	A	A
6.	Steroids Alcoholic extract+1 ml $CHCl_3$+ dropwise conc. H_2SO_4, shake.	Red colour after standing	A	A
7.	Phlobatanins 0.5 ml aq. extract+ boil with 1 ml HCl	Precipitation	P	A
8.	Saponins 2 g powder + boilin water,filter. filtrate+olive oil.	President form/ Emulsion	A	P
9.	Phenolic compounds Extract+ dil. $FeCl_3$	Violet coloured ppt	P	A

Key:- P- positive (+), A – Negative (-)

Table 6: Physicochemical Test

Sr. No.	Name of test	Tulsi oil
1	Specific Gravity	0.88
2	Acid Value	2.01%
3	Saponification Value	37.8%
4	Peroxide Value	4 meq

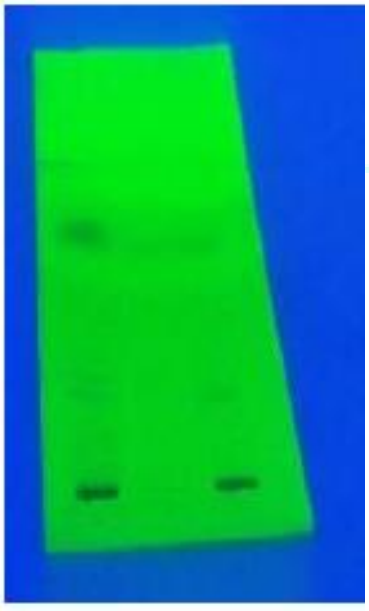
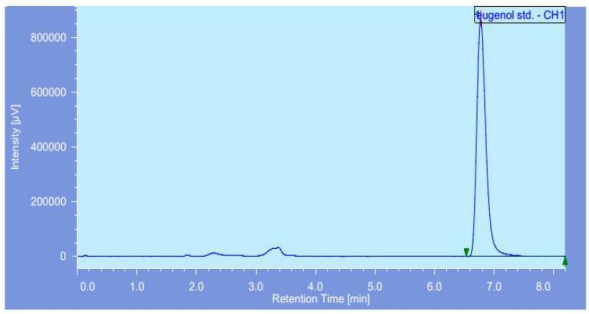
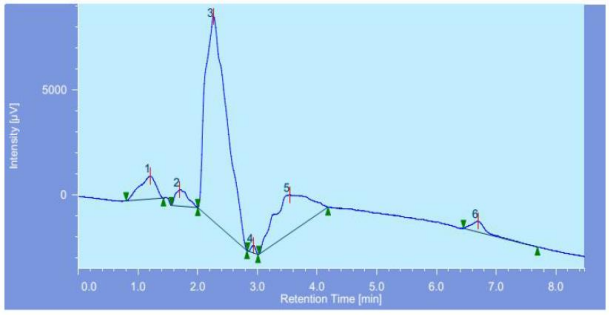


Fig.1 High Performance Thin Layer Chromatography:-

KEY :- 1) Methi 2) Tulsi 3) Formulation





Peak Name	CH	tR [min]	Area [µ V sec]
Std Eugenol	1	6.775	9310348
Tulsi oil	1	6.7	86654

Fig 2: HPLC Chromatogram

CONCLUSION:

It is very essential to assess quality control parameters of herbal medicines so as to maintain the uniformity and reproducibility in their production and to maintain its efficacy. Ayurvedic classical preparation, Tulasi oil was prepared at laboratory level and has been screened for different quality control parameters using the various modern scientific quality parameters. Chromatographic analysis plays a key role in assessing the marker compound which is very important in checking shelf life studies of herbal medicines. The results obtained can be used as reference while setting the pharmacopoeial standards for Tulasi oil to ensure the quality of the medicine. Stability studies as per different temperature conditions and for longer period are need to be performed as per the standard guidelines.

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