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Original Article

Standardization and Antioxidant Study of a Marketed Multi-component Herbal Drop of Sidh Tulsi Oil with Respect to its Active Constituents

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ABSTRACT

The current study aimed to standardize a commercial formulation of Sidh Tulsi oil and its constituent oils of various Ocimum species, including Ocimum sanctum, Ocimum basillicum, Ocimum gratissimum, Ocimum canum, and *Ocimum citriodorum*. After evaluating the physicochemical, pharmaceutical parameters, and the phytochemical study found that marketed formulations and their oils contained significant amounts of secondary metabolites, primarily polyphenols, flavonoids, tannins, triterpenoids, carbohydrates, and alkaloids. The formulation was then subjected to HPLC analysis, and it was obtained that the oil formulation showed a Retention Time which is comparable with the standard (Eugenol). The HPLC analysis of the marketed formulation of Sidh Tulsi oil and and its raw material oils confirmed the presence of Eugenol, which was found to be 3.55% (Sidh Tulsi oil), 32.54% (Ocimum sanctum oil), 4.11% (Ocimum basillicum oil), 13.11% (Ocimum gratissimum oil), 3.86% (*Ocimum canum* oil) and in vitro antioxidant study of marketed formulation and its oils showed a potential activity of the formulation. In vitro methods such as total antioxidant capacity, DPPH scavenging, and H2O2 scavenging methods were used. The chromatogram of seven compounds was obtained by GC-MS analysis of Sidh Tulsi Oil. The major compounds are identified as eugenol, glycerol, and 6-bromocoumarin-3-carboxylic acid.

Keywords: Chrmatography study, GC-MS, anti-oxidant study Ocimum species, Phytochemical study, Quantitative estimation.

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INTRODUCTION

In underdeveloped nations, between 75 and 80 per cent of the global population still receives their primary medical treatment from herbal medicine.[1] It is not a novel concept to cure animals using medicinal herbs. Plants were the initial source of many of the active ingredients found in drugs and medications. Additionally, many developing nations continue to use medicinal plants regularly. In developed nations especially, there is a resurgence of interest in using plants to treat humans, animals, and pets because many people think that plants are more affordable, more natural, and easier to make at home than artificially manufactured drugs.

Herbal and traditional medicines follow this pattern: medicinal plants are utilised for therapeutic purposes as well. They can be employed whole to produce tiers or poultices. Utilising the plant in its whole state has the benefit of allowing its many parts to work in concert to achieve the intended result.[2] Out of the 20,000 medicinal plants in the globe, India is home to around 15% (3000-3500). Plants have been used medicinally for a very long time. Writings from as far back as 4000-5000 B.C. suggest that plants have been used medicinally. numerous medicinal systems used in India and regional health customs have long relied on a vast array of plants to cure human ailments.[3]

Herbal medication technology encompasses all the processes involved in turning plant resources into medicinal products. Standardisation and quality control, together with the appropriate fusion of contemporary scientific methods and traditional wisdom, will continue to be crucial.[4] Herbal medicine is a scientific field that focuses on using plant-based remedies to treat illnesses. It is sometimes referred to as phytomedicine or botanical medicine. Phytotherapy has recently been presented as a more precise synonym for botanical or herbal medicine. Phytopharmacotherapy is the term used to describe the use of herbal medicine in the treatment of sickness. Furthermore, nutritional supplements have been made with herbal therapeutic items. Understanding complementary and alternative medical systems, such as Ayurveda, as well as other fields of the life sciences, such as botany, pharmacognosy, and establishing a set of standards or intrinsic features, consistent parameters, and definite qualitative and quantitative values that convey an assurance of quality, effectiveness, safety, and repeatability is the process of standardising herbal medicines. It is the process by which technical standards are created and approved. Experimentation and observation are used to determine specific criteria, which then inform the process of prescribing a set of qualities that the specific herbal medication possesses. Standardisation is therefore a tool used in quality control.[5]

MATERIALS AND METHODS

All chemicals and reagents used were analytical grade. Standard marker procured from Sigma-Aldrich Merck Bangalore, India. Procurement of raw materials and Marketed Formulation from Shree Baidyanath

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Ayurved Bhawan Pvt. Ltd., Great Nag Road, Nagpur (Maharashtra). Formulation and raw materials selected for research work were marketed as multicomponent herbal drops of Sidh Tulsi and its raw materials of *Ocimum sanctum* oil, *Ocimum basillicum* oil, *Ocimum gratissimum* oil, *Ocimum canum* oil, *Ocimum citriodorum* oil.

Physicochemical properties

Marketed Sidh Tulsi oil and its raw materials of *Ocimum sanctum* oil, *Ocimum basillicum* oil, *Ocimum gratissimum* oil, *Ocimum canum* oil, *Ocimum citriodorum* oil. Carried out the physiochemical evaluation such as pH measurement.[6] Specific gravity.[7] Determination of viscosity.[8] by doing Rheology of oil formulations was studied by Brookfield digital viscometer at various speeds. The measurement was done in triplicate and average values were calculated. Determination of Spreadability.[9] Refractive Index.[10] Determination of Acid Value (for oil) and Determination of Saponification Value.[11]

Preliminary phytochemical screening

For a wide range of chemicals including, amino acids, Protein, Carbohydrates/ Sugars, Volatile oil, Mucilage, Sterols, Alkaloids, Saponins (Foam test), Tannins Test for Flavonoids (Shinoda test) etc., the plant may be thought of as a biosynthetic laboratory.[12] These substances are known as secondary metabolites, and it is via them that medicinal effects are produced. A series of chemical tests were conducted on each extract to determine if primary and secondary metabolites were present or absent.

Quantitative estimation of phytoconstituents

According to the preliminary phytochemical screening findings. Using conventional techniques, the identified phytoconstituents were quantitatively determined by using total phenolic content[13] total carbohydrate content,[14] total flavonoid content,[15] and total tannin contents.[16]

Chromatographic Analysis

U.V. analysis of scraped spots by using The ultraviolet analysis of the scraped spots was done at the short wavelength, HPLC Quantitative Analysis: HPLC (UFLC Shimadzu, SPD-M20A with PDA Detector and Optimization of chromatographic conditions: Spinchotech Pvt. Ltd., Enable's C18 analytical column was used for the chromatographic investigations. After preparing the mobile phases in closed solvent bottles, they were sonicated for around 20 min. With a flow rate of 1.0 mL/min, a gradient mobile phase comprising methanol: water (60:40) yielded the highest peak resolution at a shorter retention period for ethanol. After it was discovered that eugenol had a maximum absorption wavelength of 215 nm, this wavelength was chosen as the analysis's detection wavelength.[17]

GC-MS analysis and identification

Gas chromatography-mass spectrometric analysis was performed using an Agilent 7890 GC System, USA, which was interfaced with an electron-ion spray mass detector and had a DB-5 fused silica column (30 m x 0.25 mm, film thickness 0.25 m). The parameters for the analysis were as follows: helium (2 mL/min) was the carrier gas; the temperature programme began at 50 C (5 min) and escalated to 280 C (40 min); the ionisation energy was 70 eV; the scan period was 2-42 min; and the mass range was used. By comparing the fragmentation pattern and retention index data with the standards using the WILEY 139. library and NIST 12. library, the components were found.[18]

In vitro antioxidants assay

Free radical scavenging activity: The free radical scavenging activity of plant extract/fractions was measured by the 1, 1-Diphenyl-2-Picryl-Hydrazil (DPPH) method.[19] **Scavenging of hydrogen peroxide:** The scavenging activity of plant extract/fractions was evaluated by the method described.[19] All readings were performed in triplicates and the percentage inhibition was calculated using statistical data.

RESULTS AND DISCUSSION

The total antioxidant capacity of the commercial formulations of Sidh Tulsi oil (61.36±1.64 mcg/mL), *Ocimum sanctum* oil (57.95±1.20), *Ocimum basillicum* oil (59.31±1.25), *Ocimum gratissimum* oil (77.04±67), Ocimum canum oil (62.27±2.7), and Ocimum citriodorum oil (57.72±1.5) were calculated using a linear regression equation Figure 1. The results were expressed as the number of equivalents of ascorbic acid that were discovered. By giving DPPH an electron or hydrogen, a sample can decrease it, which indicates how effective it is in scavenging free radicals.

The *Ocimum sanctum* oil (196.152±22.245 mcg/mL), *Ocimum basillicum* oil (187.182± 25.163 mcg/mL), *Ocimum gratissimum* oil (191.431±431 mcg/mL), *Ocimum canum* oil (181.64±28.43 mcg/mL), and *Ocimum citriodorum* oil (171.541±21.63 mcg/mL) exhibit the free radical scavenging activity (IC_{50} of value) Figure 2. The ascorbic acid standard's IC_{50} value was compared to the hydrogen peroxide scavenging potential (IC_{50} value) of several commercial formulations, including SIdh Tulsi Oil (245.991±10.021 mcg/mL), *Ocimum basillicum* oil (256.267±16.341 mcg/mL), *Ocimum gratissimum* oil (291.168±15.345 mcg/mL), *Ocimum canum* oil (298.159±20.261 mcg/mL), and *Ocimum citriodorum* oil (267.457±10.021 mcg/mL) Figure 3.

Efforts were undertaken in the current study to create a quality control profile of the commercial formulation of Sidh Tulsi oil and each of its constituent oils (raw materials). A variety of Ocimum extracted oil species are combined in the marketed oil mixture. We can establish standards for a certain drug by estimating the numerous qualitative and quantitative characteristics that were assessed throughout the study. These standards may then be used for the identification and characterisation of that specific drug or formulation.

These guidelines assist in ensuring the quality and purity of the medicine in question as well as its formulation, preventing it from being tainted by low-potency drugs from the same genus or other species (Shanbhag, 2008). The current study examines the formulation of marketed oils and the standardisation of each oil's active ingredients. Colour, odour, taste, soluble, appearance, density, specific gravity, viscosity, spreadability, refractive index, acid value, and saponification value are among the physicochemical parameters that are determined for an oil formulation Tables 1 and 2. These parameters were found to be within the bounds specified by the Indian Pharmacopoeia.

The results of the phytochemical screening revealed that every oil formulation included total phenol, tannin, triterpenoids, carbohydrates, and flavonoids Tables 3 and 4. TLC facilitates the examination of reactions, the identification of compounds in mixtures, the separation of multiple components in pharmaceutical formulations, and the identification and separation of colour, preservatives, and sweeteners in the cosmetics industries (Kasture *et al.*, 2004). TLC was used in this

Physicochemical Evaluation

Table 1: Organoleptic evaluation.

Sl. No.	Parameters	Sidh tulsi oil	Ocimum sanctum oil	Ocimum basillicum oil	Ocimum gratissimum oil	Ocimum canum oil	Ocimum citriodorum oil
1.	Colour	Whitish- pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
2.	Odor	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
3.	Taste	Pungent	Pungent	Pungent	Pungent	Pungent	Pungent
4.	Solubility	90% all alcohols	90% all alcohols	90% all alcohols	90% all alcohols	90% all alcohols	90% all alcohols
5.	Appearance	Clear Liquid	Clear Liquid	Clear Liquid	Clear Liquid	Clear Liquid	Clear Liquid

Table 2: Physical Evaluation.

Sl. No.	Parameters	Sidh tulsi oil	Ocimum sanctum oil	Ocimum basillicum oil	Ocimum gratissimum oil	Ocimum canum oil	Ocimum citriodorum oil
1.	pH	6.1	5.6	5.8	5.2	5.3	5.4
2.	Density	1.703	1.501	1.431	0.938	o.9137	0.961
3.	Specific gravity	0.9416	0.9255	o.9325	0.9541	0.9374	0.9562
4.	Refractive Index at 20 ˚C	1.502	1.502	1.502	1.412	1.412	1.382
5.	Spreadability	Good	Good	Good	Good	Good	Good
6.	Viscosity (cps)	30542	29089	29745	28734	29572	29208

Qualitative Estimation of the oil

Table 3: Phytochemical Screening of oils.

Sl. No.	Parameters	Sidh tulsi oil	Ocimum sanctum oil	Ocimum basillicum oil	Ocimum gratissimum oil	Ocimum canum oil	Ocimum citriodorum oil
1.	Triterpenoids	+	+	+	+	+	+
2.	Tannins	+	+	+	+	+	-
3.	Flavonoids	+	+	+	+	+	-
4.	Carbohydrate	+	+	+	+	+	-
5.	Steroids	-	-	-	-	-	-
6.	Alkaloids	+	+	+	+	+	-
7.	Proteins	+	+	+	+	+	-
8.	Glycosides	-	-	-	-	-	-

Quantitative Estimation of Oils

Table 4: Quantitative estimation.

Parameter	Sidh tulsi oil	Ocimum sanctum oil	Ocimum basillicum oil	Ocimum gratissimum oil	Ocimum canum oil	Ocimum citriodorum oil
Total Phenol Content (mg/g) Eq. Gallic Acid	62.91±0.20	56.83±0.25	47.54±0.15	51.62± 0.25	43.72±0.20	29.24±0.15
Total Tannin Content (mg/g) Eq. Tannic Acid	35.81±0.25	41.83±0.15	32.75±0.20	36.72±0.15	33.61±0.20	- 32.45±0.20
Total Flavonoid Content (mg/g) Eq. Quercetin	47.91±0.15	51.84±0.20	39.24±0.25	44.31±0.22	34.29±0.25	37.45±0.25
Total Carbohydrate content (mg/g) Eq. Dextrose	1.20±0.15	3.85±0.20	2.82±0.25	3.29±0.15	1.78±0.15	33.45±0.20

Chromatography study: a) Thin Layer Chromatography.

Table 5: **TLC confirmation with standard.**

Sample	Solvent system	Spraying reagent	No. of Spots	Retention Factor (Rf)
Std. Eugenol	Toulene:Ethyl Acetate 7:3	Anisaldehyde sulphuric Acid	1	0.65
Sidh tulsi oil	Toulene:Ethyl Acetate 7:3	Anisaldehyde sulphuric Acid	2	0.61, 0.42
Ocimum sanctum oil	Toulene:Ethyl Acetate 7:3	Anisaldehyde sulphuric Acid	2	0.63, 0.45
Ocimum basillicum oil	Toulene:Ethyl Acetate 7:3	Anisaldehyde sulphuric Acid	2	0.59, 0.38
Ocimum gratissimum oil	Toulene:Ethyl Acetate 7:3	Anisaldehyde sulphuric Acid	2	o.60, 0.41
Ocimum canum oil	Toulene:Ethyl Acetate 7:3	Anisaldehyde sulphuric Acid	2	0.58, 0.36

In vitro Antioxidant Activity

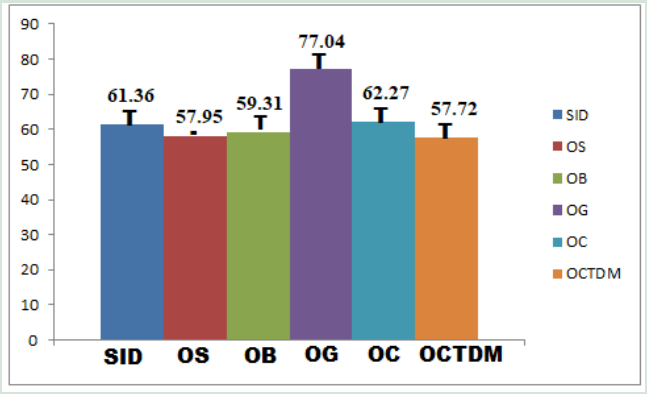


Figure 1: Total Antioxidant Capacity.

Hydrogen peroxide assay

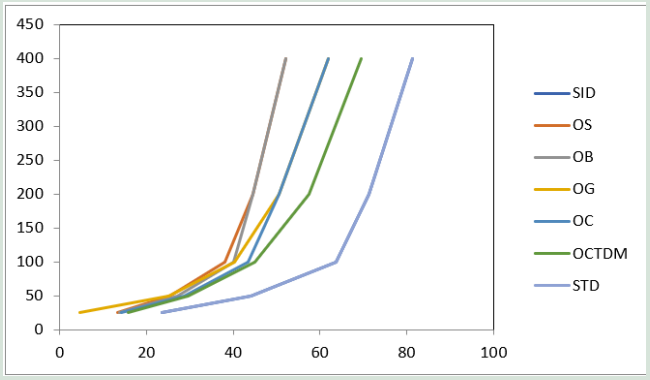


Figure 3: H_2O_2 Scavenging Method.

DPPH Assay

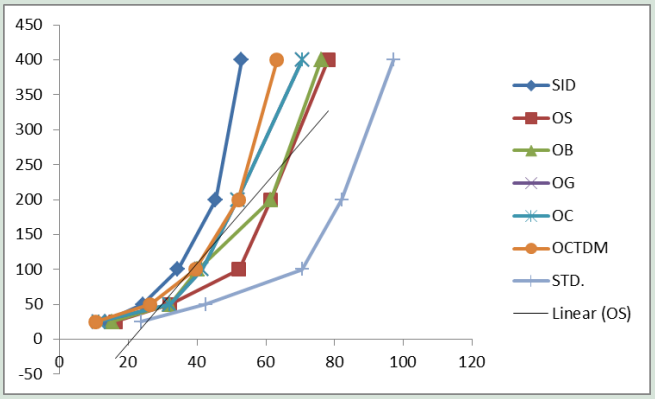


Figure 2: DDPH Scavenging Method.

High-Performance Liquid Chromatography

Eugenol

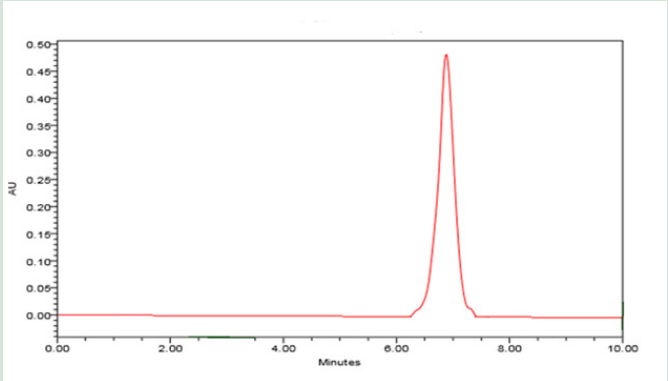


Figure 4: HPLC Chromatogram for Standard Eugenol.

Sidh Tulsi Oil

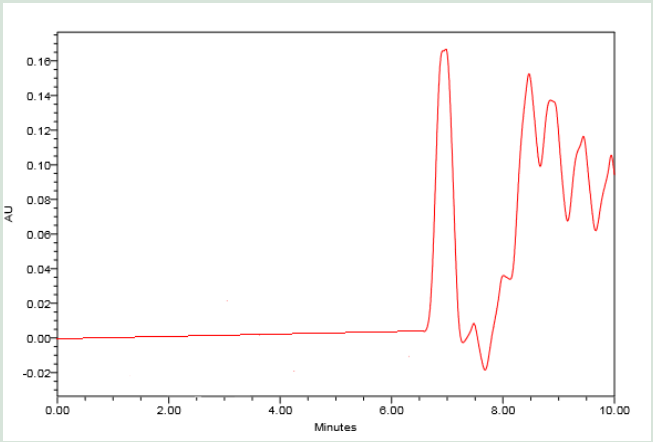


Figure 5: HPLC Chromatograph of Sidh Tulsi Oil.

Ocimum sanctum oil

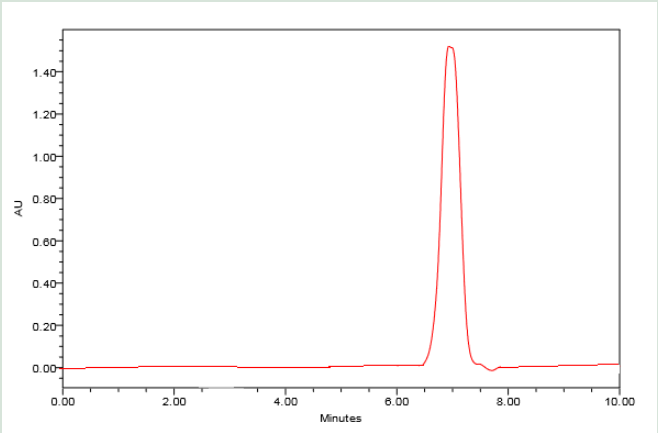


Figure 6: HPLC Chromatogram for *Ocimum sanctum* oil.

Ocimum basillicum Oil

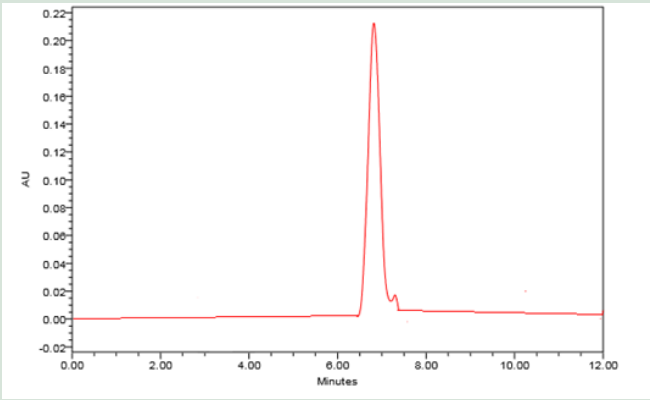


Figure 7: HPLC Chromatogram for *Ocimum basillicum* Oil.

HPLC Chromatogram for Ocimum gratissimum Oil

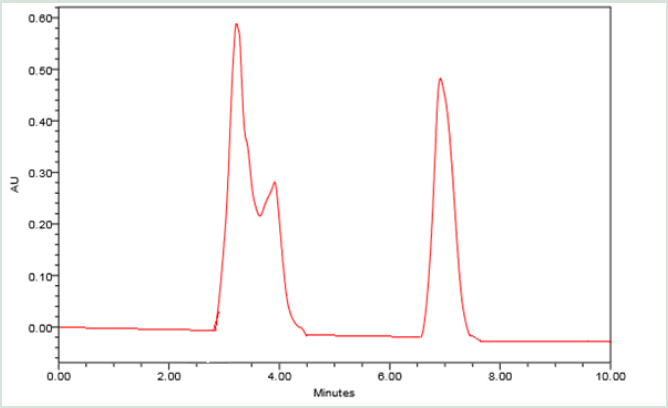


Figure 8: HPLC Chromatogram for *Ocimum gratissimum* Oil.

HPLC Chromatogram for *Ocimum canum* Oil

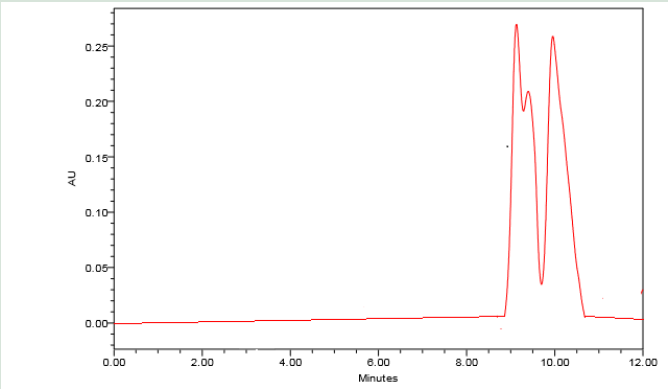


Figure 9: HPLC Chromatogram for *Ocimum canum* Oil.

investigation to identify the various ingredients that were included in the formulations. Since the formulations are mixtures of several extracted oils from Ocimum species, the TLC revealed distinct fingerprint patterns for the formulations in the solvent system of toluene: ethyl acetate (7:3) Table 5.

Following HPLC examination of the formulations, it was discovered that the oil formulation had a retention time that was equivalent to the standard (Eugenol) Figure 4. *Ocimum sanctum* oil (6.918), *Ocimum basillicum* oil (6.820), *Ocimum gratissimum* oil (6.918), *Ocimum canum* oil (6.967), and Sidh Tulsi oil (6.976) all have HPLC quantification Table 6. Ocimum citriodorum oil did not have any. distinct fingerprint patterns of the solvent systems in Toluene: Ethyl Acetate (7:3) Figures 5-9.

The herb's numerous medicinal benefits are believed to be mainly attributed to eugenol. Numerous research have examined holy basil (*O. sanctum*), and the active component eugenol has shown promise as a strong adaptogenic agent that can treat a variety of illnesses (Hahn *et al.*, 2013). The study also evaluated the oils' potential for antioxidants and how they were formulated. Using *in vitro* techniques including total

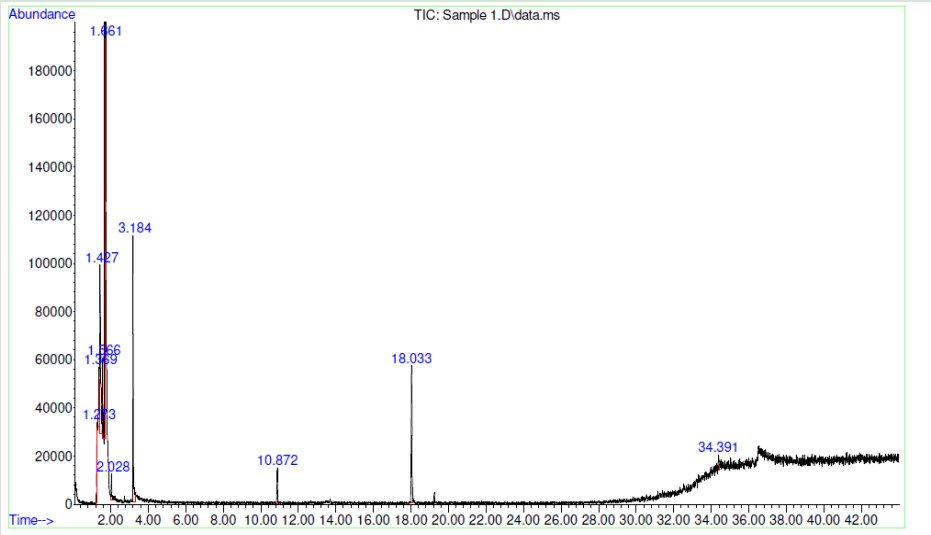


Figure 10: GC-MS Chromatogram of Sidh Tulsi Oil.

Table 6: Retention Time, Conc., % of Eugenol present in Sidh Tulsi oil (Marketed Formulation) and its Raw Materials of oils.

Sl. No.	Name of Ingredients	Concentration	Retention Time	Area (UV sec)	(% OF Eugenol)
1.	Standard Eugenol	0.1 mg/mL	6.882	8983994	100%
2.	Sidh Tulsi oil	1.01 mg/mL	6.976	32255732	3.55%
3.	*Ocimum sanctum* oil	1.16 mg/mL	6.918	33995154	32.54%
4.	*Ocimum basilicum* oil	1.01 mg/mL	6.820	3732063	4.11%
5.	*Ocimum gratissimum* Oil	1.05 mg/mL	6.918	12393625	13.11%
6.	*Ocimum canum* oil	0.98 mg/mL	6.967	3403922	3.86%
7.	*Ocimum citrodenum* Oil	1.01 mg/mL	-	-	-

antioxidant capacity, DPPH scavenging, and H_2O_2 scavenging, the oil formulation was shown to have strong antioxidant activity.

Through GC-MS analysis, the chromatogram of seven components was obtained from Sidh Tulsi Oil Figure 10. The principal constituents are recognised as 6-bromocoumarin-3-carboxylic acid, glycerol, and eugenol.

CONCLUSION

The goal of the current study was to standardise a market-ready formulation of Sidh Tulsi Oil as well as its constituent oils from several Ocimum species, such as *Ocimum citriodorum*, *Ocimum sanctum*, *Ocimum basillicum*, and *Ocimum gratissimum*. The marketed formulation oil and each of its constituent oils had their physicochemical and pharmaceutical properties assessed, and the results showed that both were within the recommended bounds according to the standard values listed in the API. A phytochemical analysis identified many compounds that were discovered to be present in significant amounts in both the individual oils and the marketed formulation, including polyphenols, flavonoids, tannins, triterpenoids, carbohydrates, and alkaloids. Eugenol was found to be present in the marketed formulation of Sidh Tulsi oil and its raw material oils by HPLC analysis. It was found to be present in 32.54% of *Ocimum sanctum* oil, 4.11% of *Ocimum basillicum* oil, 13.11% of *Ocimum gratissimum* oil, 3.86% of *Ocimum canum* oil. An *in vitro* antioxidant investigation of the commercial formulation and each of its constituent oils indicated the formulation's possible efficacy. The current study's findings indicate that, in terms of their antioxidative capability, commercial oil formulations and individual oils made from Ocimum species oils were determined to be the most effective. Therefore, the current commercial formulation of Sidh Tulsi oil has the potential to be a powerful tool for treating a wide range of illnesses in the future.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; **HPLC:** High-Performance Liquid Chromatography; **DPPH:** 1,1-Diphenyl-2-picrylhydrazyl; **H_2O_2:** Hydrogen Peroxide; **UFLC:** Ultra-Fast Liquid Chromatography; **PDA:** Photodiode Array; **UV:** Ultraviolet; **TLC:** Thin-Layer Chromatography; **R_f:** Retention Factor; **API:** Ayurvedic Pharmacopoeia of India; **cps:** Centipoise, the unit used for measuring viscosity; **IC_{50}:** Half-Maximal Inhibitory Concentration, used to express antioxidant potential.

CONFLICT OF INTEREST

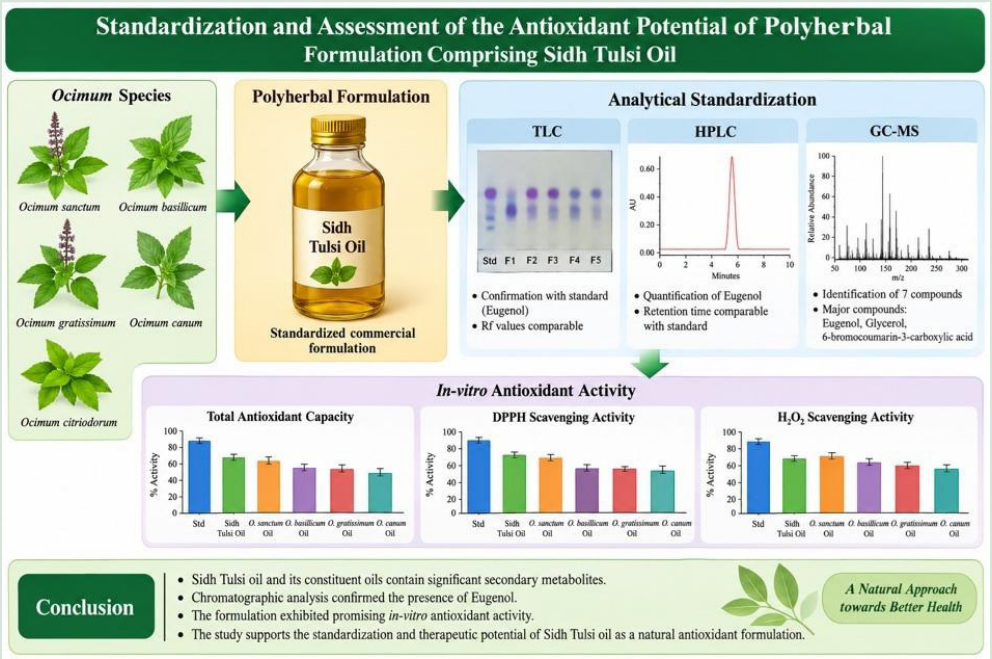
The authors declare that there is no conflict of interest.

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GRAPHICAL ABSTRACT



SUMMARY

The study focused on the quality assessment and standardization of the commercially marketed Sidh Tulsi oil and individual Ocimum oils incorporated into the formulation. The oils obtained from different Ocimum species can be diverse in their characteristics and chemical phytoconstituents. It is essential to evaluate the individual oils as well as the finished product to ensure the quality and authenticity of the formulation. In this study, both the individual oils and the marketed preparation were examined for their organoleptic and physicochemical characteristics. The parameters such as colour, odour, taste, appearance, solubility, density, specific gravity, viscosity, spreadability, refractive index, acid value, and saponification value were determined. The observations made were found to be in compliance with the pharmacopoeial requirements indicating that the oils possessed satisfactory physicochemical characteristics. The phytochemical screening of the samples revealed the occurrence of various classes of constituents including phenolic compounds, tannins, flavonoids, triterpenoids, and carbohydrates. Further, the TLC examination with Toluene and Ethyl acetate (7:3) as the developing solvent system yielded characteristic separation pattern for the individual Ocimum oils and the marketed Sidh Tulsi oil. The observed TLC profiles can serve as useful reference patterns to compare and identify the oils. The fingerprints generated can also aid in the routine quality assessment by helping in the identification of variations in the raw materials and detecting any substitution or adulteration involving oils from different Ocimum species.

HPLC analysis was carried out using eugenol as a marker compound. Chromatographic profiles for O. sanctum, O. basilicum, O. gratissimum, O. canum, and Sidh Tulsi oil, showed retention times of 6.918, 6.820, 6.918, 6.967, and 6.976 min, respectively. Eugenol was not detected in O. citriodorum under the analytical conditions used. The presence and variety of eugenol among the tested oils demonstrate its potential as a marker for the quality assessment of selected Ocimum-based formulations. The antioxidant properties of the oils were evaluated using total antioxidant capacity, DPPH radical-scavenging activity, and hydrogen peroxide-scavenging activity. The formulation displayed remarkable antioxidant potential in the tested in vitro models, which could be attributed to the presence of phenolic and other bioactive constituents.

GC–MS analysis of Sidh Tulsi oil identified seven chromatographically detectable constituents. Of the detected compounds, eugenol, glycerol, and 6-bromocoumarin-3-carboxylic acid were reported as prominent constituents. The chromatographic and phytochemical findings, along with the physicochemical evaluation and antioxidant assessment, provide a complete profile of the formulation. Overall, the study shows that the combined application of physicochemical testing, phytochemical screening, TLC fingerprinting, HPLC marker analysis, antioxidant evaluation, and GC–MS profiling can provide a valuable methodology for the standardization and quality assessment of Sidh Tulsi oil. The established features can serve as reference parameters for authentication, quality control, consistency evaluation and detection of possible adulteration or substitution in available commercially Ocimum-based herbal oil formulations.

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