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Comparative study on phytochemical analysis of active ingredient of *Ocimum sanctum* and *Glycyrrhiza glabra* leaf extracts

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ABSTRACT

The objectives of this study includes the collection of *Ocimum sanctum* and *Glycyrrhiza glabra* leaves for phytochemical extraction and preparation of solvent (aqueous) extraction from them for phytochemical analysis. Moreover it further involves the extraction of carbohydrates, alkaloids, glycosides and secondary metabolites from the leaves extract for phytochemical screening.On the basis of it the comparative study of *Ocimum sanctum* and *Glycyrrhiza glabra* was done.

Key words*: Ocimum sanctum, Glycyrrhiza glabra Phytochemical analysis, Phytochemical screening.*

Introduction

Herbal medicine refers to using leaves of plants, flowers, seeds and roots of plants for medicinal purpose which is also known as Botanical Medicine and Phytomedicine (Nikhil *et al.,* 2021). Due to the medicinal, nutritional and spiritual properties, Tulsi can be found in every house of Indian Subcontinent. It is usually also known as "Holy Basil or Sacred Basil" (Badore *et al*., 2020).

Table 1. Scientific Classification

Particular	Tulsi	Mulhethi
Kingdom	Plantae	Plantae
Order	Lamiales	Fabales
Family	Lamiaceae	Fabaceae
Genus	Ocimum	Glycyrrhiza
Species	sanctum	Glabra
Botanical name	Ocimumsanctum	Glycyrrhiza glabra

Types of Tulsi

Three main types of tulsi are- (Jurges *et al.,* 2018)

Rama Tulsi	Krishna Tulsi	Vana Tulsi
Green leaf Tulsi is also called as Rama Tulsi, Sri Tulsi. It is famous for its cool and mild taste. It has green leaves, light purple flowers and it is the most dominant of all evergreen plants. For religious purposes, it is often used and commonly called *Ocimum*	It has purple-colored leaves so it is named after the Hindu God Krishna, as Lord Krishna was also black in colour. It is used as a plant for its medicinal properties. It is assigned as *Ocimum uniform L.* Its leaves are short and crisp in texture. It also has clove like aroma and peppery flavor. It is more	As the name indicates, it is found in the wild. Vana Tulsi states 'forest' Tulsi. Its leaves are generally bright to light green in colour. It grows in wild and is native to various parts of Asia and North east Africa.

tenuiflorum. Due to the presence of a chemical called Eugenol, it has clove like smell. It has a distinctive fragrance.	potent than Rama Tulsi. It is a natural contraceptive and is also used to cure throat infection, respiratory and skin problems. It is also used to cure ear ache.

Glycyrrhiza glabra is a perennial herbaceous plant belonging to Fabaceae (Leguminosae) family, commonly known as licorice, mulethi, yashtimadhu, sweet wood (Sohail *et al*., 2017). It is the oldest and generally used plant in the Ayurveda medical history, as a medicine and also as a flavoring agent in confectionery items and to change unpleasant taste of other medicines (Biondi *et al*., 2005).

Phytochemistry and Bioactive Compounds

Phytochemicals are the biologically active, naturally occurring substances in plants that have protective and disease preventive properties. Recent studies on Mulhethi's roots proved that a very large number of chemical compounds are present including water-soluble and biological active complex which accounts for 40-50% of its total dry weight. This active complex is made up of polysaccharides, asparagines, triterpenes, pectins, mineral salts, simple sugars, estrogen (femalehormone), proteins, sterols, tannins, glycosi des, saponin, flavonoids, amino acids, bitters, essential oils, fat, mucilage (rhizome), resins, starch, and various other compounds. The presence of these compounds is believed to be responsible for the bioactivity of Licorice (Bradley, 1992; Hoffmann, 1990). The Tulsiplant contains many nutrients and various biologically active compounds, so its chemical composition is quite complex. Some phytochemicals includes Eugenol, Ursolic acid, Oleanolic acid, Rosmarinic acid, Carvacrol and Linalool.

Materials and Method

Plant Material-The fresh leaves of *Ocimum sanctum* and *Glycyrrhiza glabra* were collected from Tau Devilal Park, Rohtak, Haryana, India. Then tap water was used to wash the collected leaves to prevent dust and unwanted material deposited them. Further, the collected leaves were washedand dried at room temperature. After a period of 4-5 days, properly dried leaves were crushed and grinded to get the fine powder and this powder was weighed to get the extract. A clean and air tight container was used to keep the powder of leaves for extraction.

Preparation of extracts - A Soxhlet apparatus was used to prepare aqueous extract which was further collected, labelled and stored for experimental use. The residues were collected and used for testing purpose.

Analysing aqueous extract phytochemically

The aqueous extract was prepared using soxhelt apparatus which was further analyzed phytochemically for determining the presence as well absence of various phytochemical components.

Quantitative Analysis and Tests Involved in it

Extract and raw powder was utilized for qualitatively analysis to confirm the presence of chemical constituents by performing various chemical tests.

Tests for Identification of Phyto-chemicals

Phyto-chemicals	Tests
1. Carbohydrates	Molisch test
	Benedict test
2. Alkaloids	Wagner's test
	Mayer's test
3. Glycoside	Libermann Test
	Foam Test
	Legal Test
	Keller-Kiliani Test
4. Tannins	Gelatin Test
	Lead Acetate Test
	Ferric chloride Test
5. Oil	Stain Test
6. Flavanoid	Ferric Chloride Test
	Sodium Hydroxide Test
7. Steroid	Liebman & Burchard Test
	Salkowski Test
8. Protein	Minhydrin Test
	Millon's Test
9. Saponin	Foam Test

Test for Carbohydrates

2 ml extract was dissolved in 4ml distilled water and filtered using wattman's filter paper. Then filtrate obtained was utilized for mentioned tests to determine the presence of carbohydrates and glycosides.

(a) Molisch's Test: For this test 1 ml of the extract was firstly added with 2 to 3 drops of Molisch's reagent and then 2 ml conc. sulphuric acid was also

dissolved in it from sides of test tube. If brown ring appears at the junction of 2 liquids indicated confirmation of presence of carbohydrates.

(b) Benedict's Test- In this, firstly Benedict's reagent was used. Extract (150 mg) was dissolved with 2 ml of Benedict's reagent and then mixture was then heated onboiling water bath for 5 minutes. Reddish brown precipitates were detected with the confirmation of carbohydrate presence.

Test for Alkaloids

(a) Wagner's Test- In this, 5 mg of the extract was added to 0.5 ml of Wagner reagent was added to it after shaking well. Appearance of reddish brown colour confirmed the presence due to iodine forms a complex, which is insoluble.

(b) Mayer's Test- In this, 5 mg extract with 1% HCL was taken in a test tube. Further it was heated gently. Red colour mercuric iodine form in Mayer's reagent which confirms the presence.

Test for Glucosides

The extract was tested for presence of Saponin glucosides, Cardiac glucosides and Anthraquinone glucosides.

(a) **Liebermann's Test –** 5 mg Tulsi extract was mixed in 2 ml chloroform and later 2 ml of acetic acid was added to solution which was further cooled and freezed. Then, 1 ml conc. Sulphuric acid was added to it. If the colour changes from violet to green it confirms the presence of glucosides.

(b) **Foam Test-** In 100 mg of each leaves extract, 7 ml distilled water was added. Finally observation was made for saponin glycoside after shaking it well.

(c) **Legal's Test for cardiac glycosides-** To each extracts of 50 mg, 1 ml pyridine and 1 ml sodium nitro prusside solution were mixed and conclusion was done after observation.

(d) **Keller-kili NI Test for cardiac glycosides-** In 25 mg extracts, glacial acetic acid (1 ml) and Ferric chloride (1 ml) were mixed into it. Then it was heated, cooled and was transferred to test tube containing conc. sulphuric acid (2 ml). A brown coloured ring will form if there are cardiac glycosides present in the extract.

Test for Flavanoids

(a) **Sodium hydroxide Test**: 5mg leaf extracts were taken with 1 ml of 10% sodium hydroxide solution for the test. On addition of 2 ml of dilute hydrochloric acid, if the solution turns colourless it confirms presence of alkaloids.

(b) **Ferric chloride Test-** 5 mg leaf extracts were dissolved in 1ml of deionised water and 0.5 ml of dilute solution of ammonia in a test tube. After that, few drops of conc. sulphuric acid were mixed. If there is Formation of yellowish colour it confirms presence of alkaloids.

Test for Tannins

(a) **Ferric chloride Test-** 5 mg of aqueous extracts were poured with 0.5 ml of ferric chloride solution. If there is appearance of blackish colored precipitate, it indicates that tannins are present.

(b) **Gelatine Test-** For running this, 5mg extract of the leaves was added with gelatin in 1ml deionized water. White precipitates should be produced if tannins are present.

(c) **Lead acetate-** 5 mg of extract was taken and a few drops of lead acetate were added to it. If there is appearance of brown bulky precipitates, it indicates the presence of tannins.

Test For Saponins-

(a) Foam Test- 1 ml of extract was dissolved into 5ml of deionised water and shaken gently until foam was noticed. Little foam obtained were added along with 2 drops of olive oil and again shaken well. If there is production of emulsion then presence of saponins is confirmed.

Test for Oils-

(a) Stain Test- Small amount of aqueous extract of the plant leaves was spread on a filter paper. Appearance of oil stains on the filter paper indicates the presence of oil.

Test for Steroids

(a) Libeman and burchard TEST- 5 mg of extract was dissolved in 1 ml of chloroform. Later 2 drops of conc. sulphuric acid and acetic acid were dissolved in it. If there is appearance of greenish colour it indicates presence of steroids.

Test for Proteins

(a) **Mallon's Test-** In 5 mg extract, 2 ml of mallon's reagent was taken. This was further heated (5 min.). If red colour precipitate changes colour,

then it confirms the protein's presence..

(b) **Ninhydrin Test:** The extract of the plant was mixed with 2 ml of 0.2% Ninhydrin solution and was boiled for 2 min in water bath. If violet colour appears, it confirms the presence of protein.

Results

Present study deals with the presence or absence of phytochemicals of both Tulsi and Mulhethi in the aqueous medium is given below. In this study saponins, flavonoid, steroides, cardiac glycoside, alkaloids, tannins, carbohydrates, protein and oils are investigated in presence of aquoes extract. In Tulsi extract carbohydrate, alkaloid, flavinoids, oils, tannins and proteins showed positive result and negative for Cardiac glycoside, steroids and saponins. Phytochemical investigation of Mulhethi in aquoes extract gives negative results for carbohydrates and Tannins.

Quantitative Phyto-chemical Analysis

Phytochemical	Tulsi Extract	Mulhethi Extract
Carbohydrates	+	-
Alkaloid	+	+
Glycoside	-	+
Tannins	+	-
Oil	+	+
Flavinoids	+	+
Steroids	-	+
Protein	+	+
Saponin	-	+

(+) Indicates compounds presence and (-) indicates the absence of the phytochemical.

Conclusion

In conclusion, All these phytochemicals, Saponins, Flavonoids, Steroids, Cardic glycoside, Alkaloids, Phenols and Tannins make Tulsi and Mulhethi, definitely a great source of medicinal use. The presence of tannins suggests the ability of this plant to play a major role as antidiarrhoec, antinutritional, antidiarrheal hemostatic and antihemorrhoidal compound, presence of saponins reveled anti hyper cholesterol. After deep and carefully carried research using the traditional methods, it has been proven and certified that these both herbs are loaded with a lot of chemicals and heals mankind from all the problems in today's superficial and unhealthy life which will be cost-effective because the plants are freely available. This study would lead to the establishment of some compound that could be used to formulate new and more potent drug of natural origin.

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