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Study of Medicinal Plants: *Ocimum Sanctum* & *Tinospora Cordifolia*, Bioresources to Characterize Bioactive Compounds Using Soxhlet Apparatus

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study researched the phytochemical organization of two significant restorative plants - Ocimum sanctum (Tulsi) and Tinospora cordifolia (giloy). Leaf tests of O. sanctum and stem tests of T. cordifolia were gathered, cleaned, dried and ground into powder. Extractions were performed involving ethanol and hexane solvents in a Soxhlet device. Qualitative phytochemical screening tests for a variety of bioactive compounds, including proteins, carbohydrates, terpenoids, flavonoids, saponins, tannins, steroids, alkaloids, glycosides, and phenolics, were performed on the extracts. The majority of the phytochemicals tested were found in O. sanctum extracts, while fewer

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compounds were found in T. cordifolia extracts. In particular, flavonoids, tannins, alkaloids, sugars, terpenoids and proteins were distinguished in O. sanctum but not in T. cordifolia separates. The two plants showed the presence of saponins, steroids, heart glycosides and phenolics. The study sheds light on the bioactive compound profiles of these significant medicinal plants and highlights their potential for further research in the fields of drug discovery and herbal formulation development. Future possibilities for research on these plants in regions like pharmacology, nutraceuticals and maintainable assets the executives are additionally talked about.

Keywords: Ocimum sanctum (Tulsi); Tinospora cordifolia (Giloy); phytochemical analysis; bioactive compounds; soxhlet extraction; ethanol extract; hexane extract; flavonoids.

1. INTRODUCTION

"Medicinal plants grow naturally around us. Over centuries, cultures around the world have learned how to use plants to fight illness and maintain health. These readily available and culturally important traditional medicines form the basis of an accessible and affordable healthcare regime and are an important source of livelihood for Indigenous and rural populations" [1,2].

"Of the 17,000 species of higher plants in India, produce is known for medicinal uses. This proportion of medicinal plants is the highest proportion of plants known for their medical purposes in any country of the world for the existing flora of that respective country. Ayurveda, the oldest medical system in the Indian sub-continent, has alone reported approximately 2000 medicinal plant species, followed by Siddha and Unani" [3].

"Often, a single medicinal plant can have multiple uses, and sometimes different parts of the same plant may be used to treat more than one disease condition. Other times, the same plant could be used as an ingredient in herbal preparations for a synergistic effect" [4].

Pharmacological importance of plants in cancer: "The use of plants and their phytochemicals to cure cancer has grown in popularity in recent years. Though some plant parts have therapeutic and chemo-preventive potential, their methods are challenging to decipher. As a result, several targeted compounds that have the potential to be utilized as anti-cancer agents have been the subject of intensive research. Many plants and plant compounds can change signalling pathways and their anti-inflammation and anti-apoptotic targets for cancer therapy. Phytochemicals with anti-cancer properties include resveratrol, allicin, lycopene, indole-3-carbinol, vitamin C, - gingerol, emodin, natural antioxidant mixture, sulforaphane, ellagic acid, myricetin, vanillin, and eugenol. They have one or more signalling routes through which they function" [5,6].

Medicinal plants: Medicinal plant (MP) refers to any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors of the synthesis of valuable drugs.

"A whole plant or plant parts may be medicinally active. Medicinal plants (MPs) are becoming very important due to their uses mainly as a source of therapeutic compounds that may lead to novel drugs" [7,8].

"Medicinal plants have been a resource for healing in local communities around the world for thousands of years. Still, it remains of contemporary importance as a primary healthcare mode for approximately 85% of the world's population, and as a source for drug discovery, with 80% of all synthetic drugs deriving from them. Concurrently, the last few hundred years have seen a prolific rise in the introduction, development, and advancement of herbal substance analysis" [9].

Uses: Medicinal plants, Tulsi, and Giloy cure several common ailments. These are considered home remedies in many parts of the country. It is a known fact that lots of consumers are using Basil (Tulsi) for making medicines, black tea, pooja and other activities in their day-to-day lives.

"Medicinal plants are considered rich resources of ingredients that can be used in drug development of pharmacopoeial, non-pharmacopoeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the world. Moreover, some plants are considered an important source of nutrition and as a result, they are recommended for their therapeutic values" [10].

Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. In many countries, different kinds of medicinal plants/ herbs are used to keep ants, flies, mice and away from homes and offices. Nowadays medicinal herbs are important sources for pharmaceutical manufacturing.

"Recipes for the treatment of common ailments such as diarrhoea, constipation, hypertension, low sperm count, dysentery and weak penile erection, piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea and fevers are given by traditional medicine practitioners very effectively" [11,12].

"A whole plant or plant parts may be medicinally active. Medicinal plants (MPs) are becoming very important due to their uses mainly as a source of therapeutic compounds that may lead to novel drugs" [13].

Bioactive compounds: "The term "bioactive" is an alternative term for "biologically active". Hence, a bioactive compound is simply a substance with biological activity. Bioactive compounds are essential (e.g., vitamins) and non-essential e.g., polyphenols, alkaloids", etc [13].

Bioactive compounds are mainly found as follows:

- Flavonoids
- Alkaloids
- Terpenoids
- Glycosides
- Tannins
- Lignin

***Ocimum sanctum*:** "Common name: Holy basil, sacred basil *Ocimum sanctum* (Tulsi) renowned as; "Queen of herbs", the legendary "Incomparable one". "The Mother medicine of nature", is regarded as one of the holiest and most cherished of the many healing and health-giving herbs of the Orient. The cultivation time is October to December. Ocimum sanctum is a herbaceous, multi-branched, annual, erect, softy hairy, 30-75 cm. high found thoroughly up to 1800m. Its leaves are elliptical, oblong, acute or obtuse, entire or serrate, pubescent on both sides, minutely gland-dotted. Inflorescence is Verticillate and flowers are purplish, close whorl in the form of racemes" [14].

Scientific classification:

- Kingdom - Plantae
- (Unranked) – Angiospermae
- (Unranked) – Eudicots
- (Unranked) – Asteroids
- Order – Lamiales
- Family – Lamiaceae
- Genus – Ocimum
- Species – *O. sanctum*

"*Ocimum sanctum* Linn. Have been recommended for the treatment of bronchitis, malaria, diarrhoea, dysentery, skin disease, arthritis, eye diseases, insect bites and so on. The *O. sanctum*. L. has also been suggested to possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic actions. Eugenol (1- hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum* L. are largely responsible for the therapeutic potential" [15].

List 1. Compound

Name of the compound	Molecular formula	MW	Peak Area %
Eugenol	$C_{10}H_{12}O_2$	164	43.88
Cyclohexane. 1,2,4- triethyl-	$C_{12}H_{18}$	162	15.31
Caryophyllene	$C_{15}H_{24}$	204	26.53
10-Heptadecen-8-yonic acid, methyl ester,(E)-	$C_{18}H_{30}O_2$	278	1.02
Cyclopentane, cyclopropylidene-	C_8H_{12}	108	1.02
Z,Z-4,16-Octadecadiene-	$C_{20}H_{36}O_2$	308	1.02
Benzene methanamine, N,N-a,4-tetramethyl-	$C_{11}H_{17}N$	163	2.04
Pentanedinitrile, 2-methyl-	$C_6H_8N_2$	108	6.12

"Although Tulsi is known as a general vitalizer and increases physical endurance, it contains no caffeine or other stimulants. The stem and leaves of holy basil contain a variety of constituents that may have biological activity, including saponins, flavonoids, triterpenoids, and tannins. In addition, the following phenolic actives have been identified, which also exhibit antioxidant and anti-inflammatory activities, Rosmarinic acid" [16,17].

Tinospora cordifolia: T. cordifolia commonly called Giloy/Guduchi is a natural herbal shrub that belongs to the moonseed family Menispermaceae. Guduchi is a large extensively spreading glabrous, perennial deciduous twine with succulent stems and papery bark which is widely found in India.

The leaves are simple, heart-shaped and bright green. Further, it is alternate, estipulate, entire and the lamina is broadly ovate 10-12 cm long and 8-15 cm broad showing multicoated reticulate venation.

"T. cordifolia (Guduchi) mainly consists of alkaloids, glycosides, steroids, aliphatic compounds, essential oils, a mixture of fatty acid, calcium, phosphorous, protein and polysaccharides" [18,19].

Scientific Classification:

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Order: Ranunculales
Family: Menispermaceae
Genus: Tinospora
Species: T. Cordifolia

Properties:

- Anti-Cancer/Anti-Tumor Activity
- Anti-Toxin Activity
- Anti-diabetic activity
- Immunomodulatory activity
- Anti-oxidant activity
- Anti-microbial activity
- Anti-hiv activity

2. MATERIALS AND METHODOLOGY

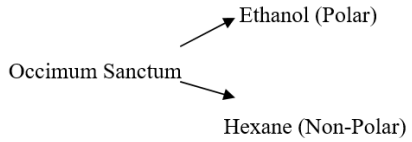
1. Collection of Sample
2. Cleaning & Drying
3. Grinding
4. Choice of Solvents
5. Extraction method
6. Phytochemicals analysis of bioactive compound

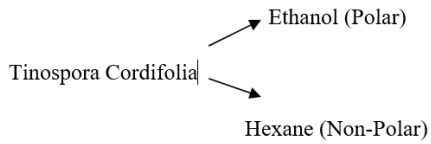
Collection of samples: The dry leaf of *Occimum sanctum* was bought from the botanical garden and stems were harvested from the garden near the location of Munsipulia Lucknow, we picked the leaf of Ocimum sanctum and stem from the *Tinospora cordifolia* because these parts of the plant contain more phytochemicals.

Cleaning & Drying: After collection, plant leaves and stems were carefully washed with tap water to remove dust, debris, and other foreign substances from the plant's surface. After cleaning, the *Ocimum Sanctum leaves* and *Tinospora cordifolia* stems were shredded and dried on blotting paper. The primary goal of drying is to eliminate water particles that have accumulated after washing. After natural drying, samples were dried for 1-2 days at 42°C in a hot air oven.

Grinding: To extract aqueous extract, the completely dried leaves and stems were ground into a fine powder with the help of a grinder machine, and then the powdery material of Tulsi and Giloy was weighed accurately and stored in a dry and clean air-tight glass container.

Choice of solvents: With the use of an instrument, we synthesized the extracts using ethanol and hexane solvents.





Extraction Method:

There are different types of extraction methods are used-

1. Maceration.
2. Soaking and water bath.
3. Soxhlet extraction.

4. Vaccume rotor.
5. Microwave-assisted extraction.

In our work, we use a soxhlet apparatus for the preparation of crude extract.

Soxhlet: An apparatus for extracting fats or other materials with a volatile solvent (thermostable compounds, lipids, vegetable oil). Continuous hot percolation is another name for it.

Principle of soxhlet: the principle of soxhlet is based on Reflux & Siphoning.

a. **Round bottom flask:** We put the solvent in it and heat it from the bottom through the mantle.
b. **Soxhlet extractor:** The evaporator tube, siphoning tube, and thimble are all part of a cylindrical tube. In the extractor chamber, a thimble (a sample packed in Whatman filter paper) is put.
c. **Condenser:** To condense the vaporized solvents, it has a water intake and a water output.

The procedure of extraction: Firstly, weigh the sample (3 gm) on a digital weighing machine. All sections of the soxhlet unit should be thoroughly cleaned and wiped. A solid material containing some of the desired components is normally placed within a thimble manufactured from Whatman thick filter paper, which is then inserted into the Soxhlet extractor's main chamber. The Soxhlet extractor is placed onto a flask containing the extraction solvent 120ml. Connect the condenser's water inlet to the faucet.

Combine all of the soxhlet components, including the lower side round bottom flask, the middle soxhlet extractor, and the upper side condenser. Adjust the appropriate boiling point for ethanol (78.37◦C) for Hexane (69◦C). Push on the button of the Soxhlet unit. “The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvents. Some of the desired compounds will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles, the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After complete extraction solvent is removed. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded” [20].

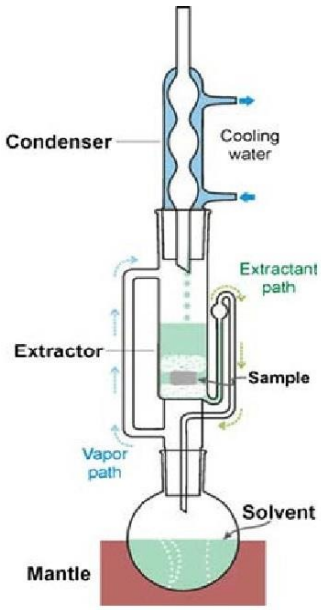
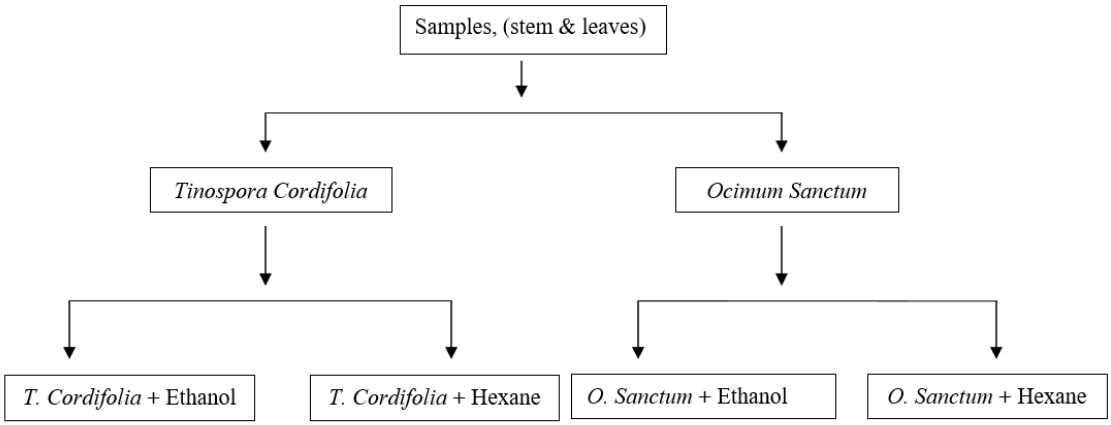


Fig. 1. Parts of Soxhlet



Flow chart 1. Phytochemicals screening

Phytochemicals Analysis of *Ocimum Sanctum* & *Tinospora Cordifolia*:-

Qualitative tests: -phytochemicals screening: "The concentrated residues from the acetone extracts were used to detect the secondary plant metabolites including alkaloids, flavonoids, steroids, saponins, glycosides, phenolics and tannins using standard methods with some modifications" [21].

Test for saponins (Frothing test): Saponins are naturally occurring chemicals found in legume plants' whole cell structure. Saponins are a complex and chemically varied collection of chemicals that get their name from their capacity to produce stable, soaplike foams in aqueous solutions.

Saponin is a bioorganic molecule derived from medicinal plants with a large molecular weight and an aglycone (water non-soluble component) nucleus containing 27 to 30 carbon atoms, as well as one or two sugar moieties (water-soluble part) containing at least 6 or 12 carbon atoms, respectively.

"Many scientists and researchers may perceive the intricacy of saponin chemistry to be a barrier to understanding the relationship between the chemical structure and its medical or pharmacological action" [22–24].

Categories of Saponins:

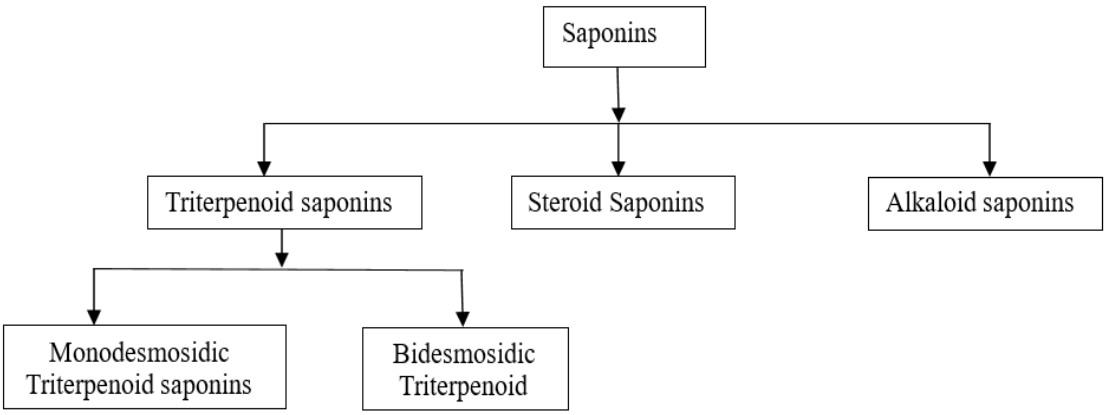


Fig. 2. Categories of saponins

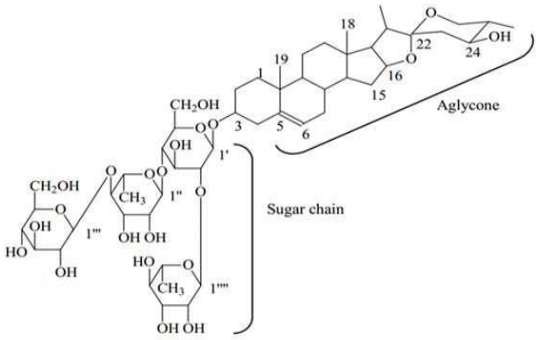


Fig. 3. The chemical structure of steroid saponin

Uses: Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning.

Test procedure: To begin, take four test tubes properly wash them with distilled water and then allow them to dry. Saponins were determined by dissolving (500µl) of crude extract in each test tube. then 3 ml hot distilled water was added, and the mixture was agitated for one minute, resulting in persistent foaming, indicating the presence of saponins [25,26].

Test for Flavonoids (Cyanidine test): Flavonoids are polyphenolic chemicals that are classified as flavones, flavanones, catechins, and lipoxygenase and are catalysed by Ca+2-ATPase, xanthine oxidase, and phosphodiesterase. They also play a function in the regulation of various anthocyanins.

Flavonoids of various kinds contain hormones such as androgens, estrogens, and thyroid hormones. In terms of pharmacological effects. Flavonoids have a wide range of pharmacological and biological functions, as well as pharmacological and biochemical effects that inhibit a variety of diseases. They appear to have a lot of therapeutic potential. Aldose reductase, cycloxygenase, and other enzymes [27,28].

Classification of flavonoids:

1. Flavones
2. Flavonols
3. Flavanones
4. Flavanonols
5. Isoflavones
6. Catechins
7. Anthocyanidins
8. Chalcones

Chemical structure of flavonoids: Their basic structure is a skeleton of diphenylpropane, namely, two benzene rings (ring A and B, See Fig. 1) linked by a three-carbon chain that forms a closed pyran ring (heterocyclic ring containing oxygen, the C ring) with benzenic A ring. Therefore, their structure is also referred to as C6-C3-C6 [29].

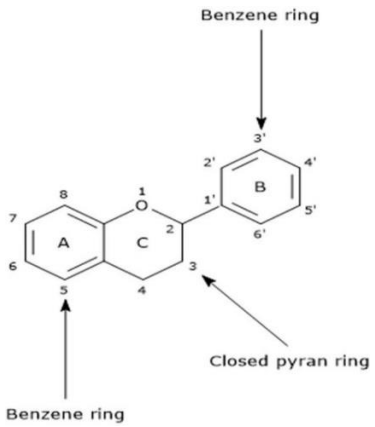


Image 1. The basic skeleton structure of flavonoids, the most abundant polyphenols in the human diet

Use of Flavonoids:

- Flavonoids aid in cellular activity regulation and the battle against free radicals that cause oxidative stress in the body. In layman's words, they aid in the effective functioning of your body while also shielding it from everyday pollutants and stressors.
- Flavonoids are potent antioxidants as well. Antioxidants aid in the fight against potentially hazardous chemicals that may enter the body. Antioxidants are produced naturally by your body, but they can also be found in dark chocolate, lentils, and a variety of fruits and vegetables.
- "One of our body's immunological responses is inflammation. Allergens, bacteria, toxins, and other irritants can cause inflammation, which can lead to a variety of unpleasant symptoms. Flavonoids may assist your body in suppressing the inflammatory response, thereby reducing the symptoms" [30,31].

Test Procedure: To begin, take four test tubes properly wash them with distilled water and then allow them to dry. Flavonoids were determined by dissolving (500µl) of crude extract & 2 ml concentrated HCL in each test tube. "A spatula full of magnesium turnings was added and the mixture was observed for effervescence. A brick red colouration observed indicated the presence of flavonoids" [32,33].

Test for steroids (Lieberman-Burchard test): "A steroid is a biologically active organic compound with four rings arranged in a specific molecular configuration. Steroids have two principal biological functions: as important components of cell membranes that alter membrane fluidity; and as signalling molecules. Hundreds of steroids are found in plants, animals and fungi. All steroids are manufactured in cells from the sterols lanosterol (opisthokonts) or cycloartenol (plants). Lanosterol and cycloartenol are derived from the cyclization of the triterpene squalene. The steroid core structure is typically composed of seventeen carbon atoms, bonded in four "fused" rings: three six-member cyclohexane rings (rings A, B and C in the first illustration) and one five-member cyclopentane ring (the D ring). Steroids vary by the functional groups attached to this four-ring core and by the oxidation state of the rings. Sterols are forms of steroids with a hydroxy group at position three and a skeleton derived from cholestane. Steroids can also be more radically modified, such as by changes to the ring structure, for example, cutting one of the rings. Cutting Ring B produces secosteroids one of which is vitamin D3" [34].

Uses:

- asthma and chronic obstructive pulmonary disease (COPD)
- hay fever.
- hives and eczema.
- painful joints or muscles – such as arthritis, tennis elbow and frozen shoulder.
- pain caused by an irritated or trapped nerve – such as sciatica.
- inflammatory bowel disease – such as Crohn's disease.
- Lupus [35,36].

Test Procedure: To begin, take four test tubes properly wash them with distilled water and then allow them to dry. Steroids were determined by dissolving (500µl) of crude extract dissolved in 0.5ml of dichloromethane to give a dilute solution and then 0.5 mL of acetic anhydride added, followed by three drops of concentrated sulphuric acid [37].

Test for tannins (Ferric chloride test): "Tannin, also called tannic acid, is any of a group of phenolic compounds in woody flowering plants that are important deterrents to herbivores and have several industrial applications. As secondary metabolites, tannins are sequestered in vacuoles within the plant cell, which protects the other cell components. They occur normally in the roots, wood, bark, leaves, and fruit of many plants, particularly in the bark of oak (Quercus) species and in sumac (Rhus) and myrobalan (Terminalia chebula). They also occur in galls, pathological growths resulting from insect attacks. Commercial tannins typically are pale yellow to light brown amorphous substances in the form of powder, flakes, or a spongy mass. They are used chiefly in tanning leather, dyeing fabric, and making ink and in various medical applications. Tannin solutions are acidic and have an astringent taste. Tannins are responsible for the astringency, colour, and some of the flavours in black and green teas" [38].

Uses:

Food industry:

9. Tannins are the secondary metabolites present in a substantial amount of plant-

based food products. Due to their positive effects on the food as antibacterial and antioxidants, they are the major constituent of foods.

10. Tannins are used as food preservatives, packaging materials, and food enhancements owe to their protective nature.

Food packaging:

11. Currently, most of the food items available in the market are wrapped in packing materials which are plastic, polyethene, low-density polyethene (LDPE) and linear low-density polyethene (LLDPE) due to their lightness, inertness, and easy availability.
12. In fact, packaging increases the shelf-life and prevents physical damage, contamination, and deterioration given environmental contaminants [39].

Test Procedure: To begin, take four test tubes properly wash them with distilled water and then allow them to dry. Tannins were determined by dissolving (500µl) of crude extract and added to a tube containing 20 mL of boiling distilled water and then boiled for an hour. A few drops of ferric chloride were added and allowed to stand for proper colour development [40,41].

Test for Alkaloids (Dragendorff's test): "Alkaloid, is any of a class of naturally occurring organic nitrogen-containing bases. Alkaloids have diverse and important physiological effects on humans and other animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine. Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. As many as one-quarter of higher plants are estimated to contain alkaloids, of which several thousand different types have been identified. In general, a given species contains only a few kinds of alkaloids, though both the opium poppy (Papaver somniferum) and the ergot fungus (Claviceps) each contain about 30 different types. Certain plant families are particularly rich in alkaloids; all plants of the poppy family (Papaveraceae) are thought to contain them, for example. The Ranunculaceae (buttercups), Solanaceae (nightshades), and Amaryllidaceous (amaryllis) are other prominent alkaloid-containing families. A few alkaloids have been found in animal species, such as the New World beaver (Castor canadensis) and poison-dart frogs (Phyllobates). Ergot and a few other fungi also produce them" [42].

Uses:

13. They may act as reservoirs for protein synthesis. They may act as protective substances against animal or insect attacks. Like hormones, they may function as plant stimulants or regulators in activities like growth, metabolism and reproduction.
14. Or they may function as detoxicating agents by methylating, condensing, and cyclizing the compounds whose accumulation might otherwise cause damage to the plant [43].

Test Procedure: To begin, take four test tubes properly wash them with distilled water and then allow them to dry. Alkaloids were determined by dissolving 2ml of crude extract and adding a few drops of Dragendorff's s reagent along the side of the test tube. Formation of an orange or orange-reddish brown precipitate indicates the presence of Alkaloids [44].

Test for cardiac glycosides: "Cardiac glycosides are found in several plants, including the leaves of the digitalis (foxglove) plant. This plant is the source of this medicine. People who eat a large amount of these leaves may develop symptoms of an overdose. Long-term (chronic) poisoning can occur in people who take cardiac glycosides every day. This can happen if someone develops kidney problems or becomes dehydrated (especially in the hot summer months). This problem usually occurs in older people. Cardiac glycosides are medicines for treating heart failure and certain irregular heartbeats. They are one of several classes of drugs used to treat the heart and related conditions. These medicines are a common cause of poisoning. Cardiac glycoside overdose occurs when someone takes more than the normal or recommended amount of this medicine. This can be by accident or on purpose" [45].

Uses:

15. They are chemical compounds responsible for the poisoning of livestock and the treatment of congestive heart failure. Extracts or latexes of cardiac glycosides plants have been applied to poison arrows in Africa, Asia, and South America for use in hunting and fighting [46].

Test Procedure: To begin, take four test tubes properly wash them with distilled water and then allow them to dry. Cardiac glycosides were determined by dissolving (500µl) of crude extract dissolved in 2 mL glacial acetic acid plus one drop of ferric chloride was mixed with a plant extract. With 1 mL of concentrated sulphuric acid, the setup was kept simple. The presence of cardiac glycosides was suggested by the emergence of violet and brownish rings below the interface, followed by the formation of a greenish ring in the acetic acid layer [47].

Test for Phenolics: "Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress. The functions of Phenolics compounds in plant physiology and interactions with biotic and abiotic environments are difficult to overestimate. Phenolics play important roles in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants. Importantly, Phenolics phytoalexins, secreted by wounded or otherwise perturbed plants, repel or kill many microorganisms, and some pathogens can counteract or nullify these defences or even subvert them to their advantage" [48,49].

Uses;

16. Phenolic compounds, ubiquitous in plants are an essential part of the human diet and are of considerable interest due to their antioxidant properties.
17. Fruits, vegetables and beverages are the major sources of phenolic compounds in the human diet.
18. The food and agricultural products processing industries generate substantial quantities of phenolics-rich by-products, which could be valuable natural sources of antioxidants [50].

Test Procedure:

- To begin, take four test tubes properly wash them with distilled water and then allow them to dry.
- Phenolics were determined by dissolving (500µl) of crude extract dissolved
- To 1 mL of the plant extract, one drop of 5 % FeCl3 (w/v) was added. The formation of a greenish precipitate indicated the presence of phenolics [51].

Test for carbohydrates (Benedict's test): Carbohydrates are the condensation products of polyhydroxy aldehydes or polyhydroxy ketones and their derivatives. They contain carbon, hydrogen and oxygen, in which the ratio of hydrogen and oxygen is generally 2: 1. Usually most carbohydrates are crystalline, water-soluble, neutral compounds, but a number of them are acidic such as pectic acid, gluconic acid, alginic acid etc.

Due to the presence of free aldehyde and ketonic moiety, the monosaccharides are highly reactive showing several chemical and physical properties, while disaccharides and polysaccharides are lacking in such properties.

Uses:

- The main role of carbohydrates in our diet is to produce energy. Each gram of carbohydrates provides us with about four calories. Carbohydrates also act as a food store. Our bodies also store carbohydrates in insoluble forms such as glycogen or starch.
- This is because these two carbohydrates are compact. Carbohydrates are also combined with nitrogen to form non-essential amino acids [52].

Test procedure: "To begin, take four test tubes properly wash them with distilled water and then allow them to dry. Carbohydrates were determined by dissolving (500µl) of crude extract dissolved. A few drops of Benedict's reagent were added to a 2 ml portion of the various extracts, boiled in a water bath for 5 min, cooled and observed for a reddish brown precipitate" [53].

Test for terpenoids (Salkowski's test): "The terpenoids constitute the largest class of natural products and many interesting products are extensively applied in the industrial sector as flavours, fragrances, and spices and are also used in perfumery and cosmetics. Many terpenoids have biological activities and are also used for medical purposes. In higher plants, the conventional acetate-mevalonic acid pathway operates mainly in the cytosol and mitochondria and synthesizes sterols, sesquiterpenes and ubiquinones. In the plastid, the non-mevalonic acid pathway takes place and synthesizes hemi-, mono-, sesqui-, and diterpenes along with carotenoids and phytol tail of chlorophyll" [54].

Uses:

- Terpene is also used to enhance skin penetration and prevent inflammatory diseases.
- Modern medication uses large scales of terpene for various treatment drugs [55].

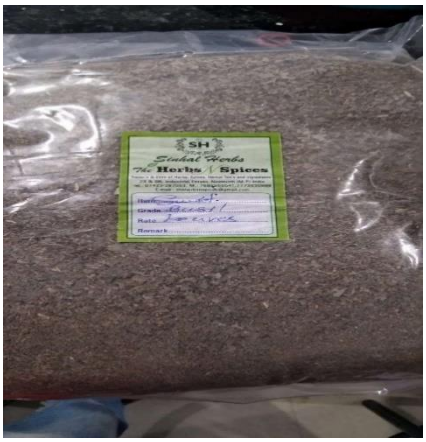
Test procedure: To begin, take four test tubes properly wash them with distilled water and then allow them to dry. Terpenoids were determined by dissolving (2ml) of crude extract treated with 1ml chloroform followed by a few drops of concentrated sulphuric acid. A reddish-brown precipitate produced immediately indicated the presence of terpenoids [56].

Test for amino acids and proteins (1% ninhydrin solution): Amino acids play several vital roles in the central metabolism of plants. Essential amino acids (EAAs), notably lysine and methionine, cannot be synthesized by humans or animals, and must therefore be acquired via food sources. “Amino acids have various prominent functions in plants. Besides their usage during protein biosynthesis, they also represent building blocks for several other biosynthesis pathways and play pivotal roles during signalling processes as well as in plant stress response. In general, pool sizes of the 20 amino acids differ strongly and change dynamically depending on the developmental and physiological state of the plant cell” [50].

Test procedure: “To begin, take four test tubes properly wash them with distilled water and then allow them to dry. 2ml of the filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1- 2 minutes and observed for the formation of purple colour” [57].

3. RESULTS & DISCUSSION

Collection of samples: *Ocimum Sanctum & Tinospora Cordifolia.*



Ocimum Sanctum

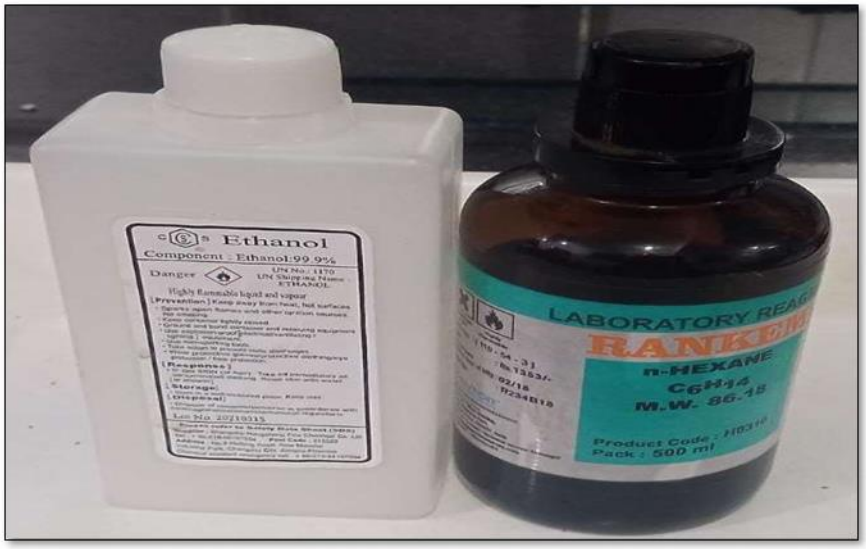
Tinospora Cordifolia

Cleaning & Drying: After washing sample shredded dried on blotting paper.

Cleaning

Drying

Choice of solvents: - We chose ethanol and Hexane for extraction procedure



Extraction: - Samples of extract were kept in a flask and stored for further chemical analysis.

(a). Saponins: Foam is present in all four test tubes, indicating the presence of saponins.

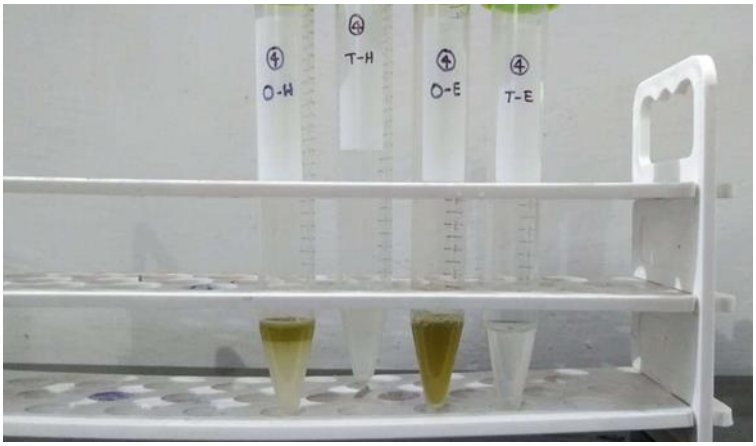


Fig. 4. Test for saponins (Frothing test)

(b). Flavonoids: The Red brick color was observed in two test tubes that contained Ocimum Sanctum+ Ethanol & Ocimum Sanctum+ Hexane

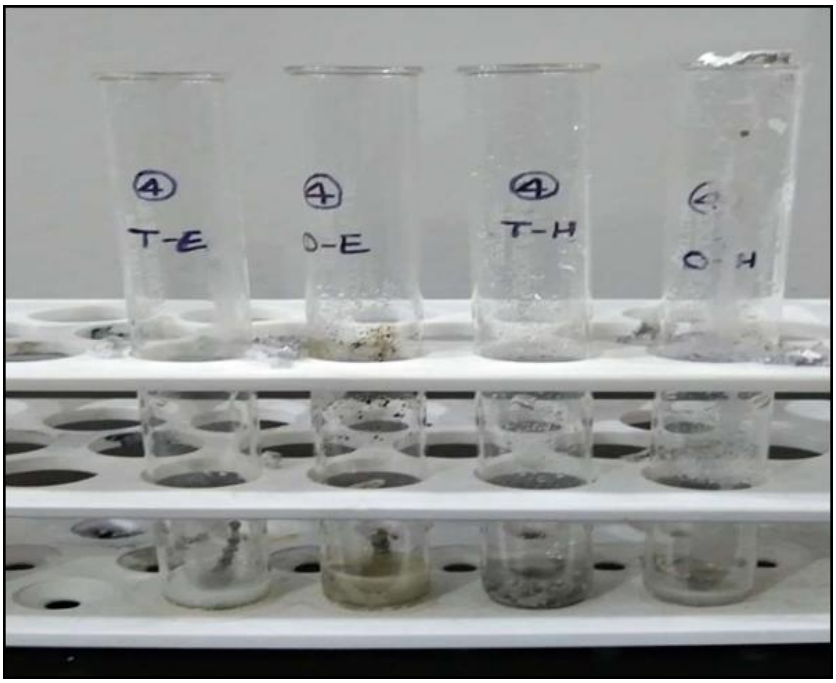


Fig. 5. Test for Flavonoids (Cyanidine test)

(c) Phenolics: In all four test tubes greenish precipitate indicated the presence of Phenolics.

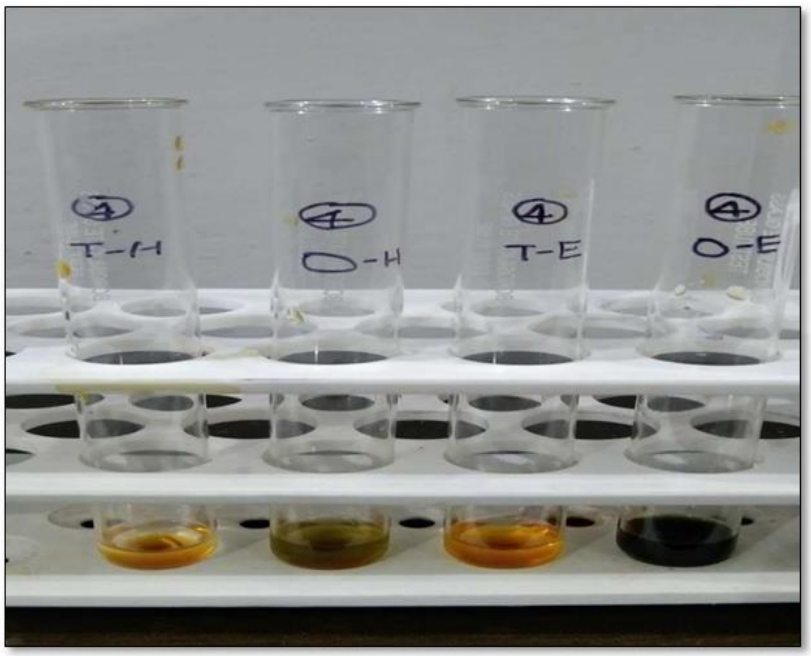


Fig. 6. Test for Phenolics

(d). Amino acids and proteins: The two test tubes of Ocimum sanctum (Ethanol & Hexane) produce purple color and T. Cordifolia produces no colour.

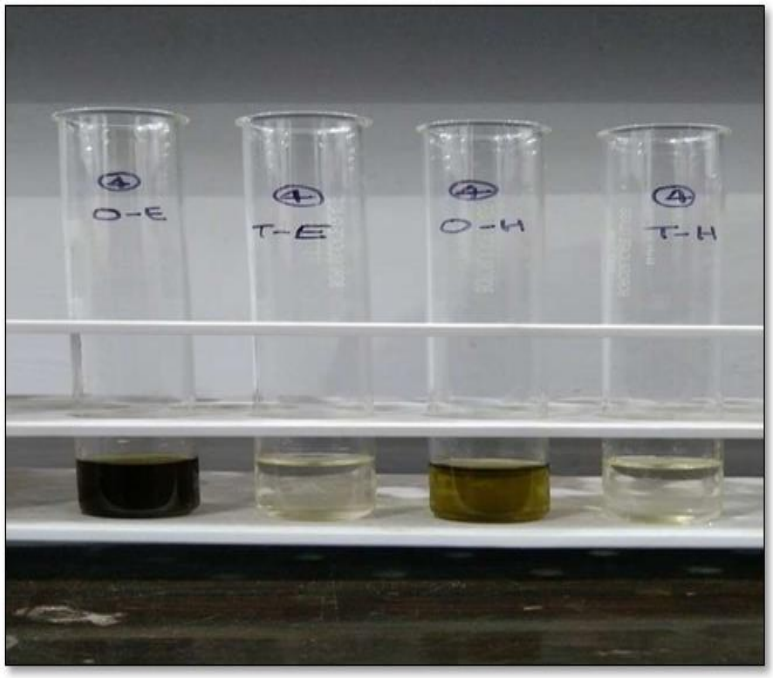


Fig. 7. Test for amino acids and proteins (1% ninhydrin solution)

Terpenoids: The two test tubes of Ocimum sanctum (Ethanol & Hexane) produces reddish brown color and T. Cordifolia produces no colour.

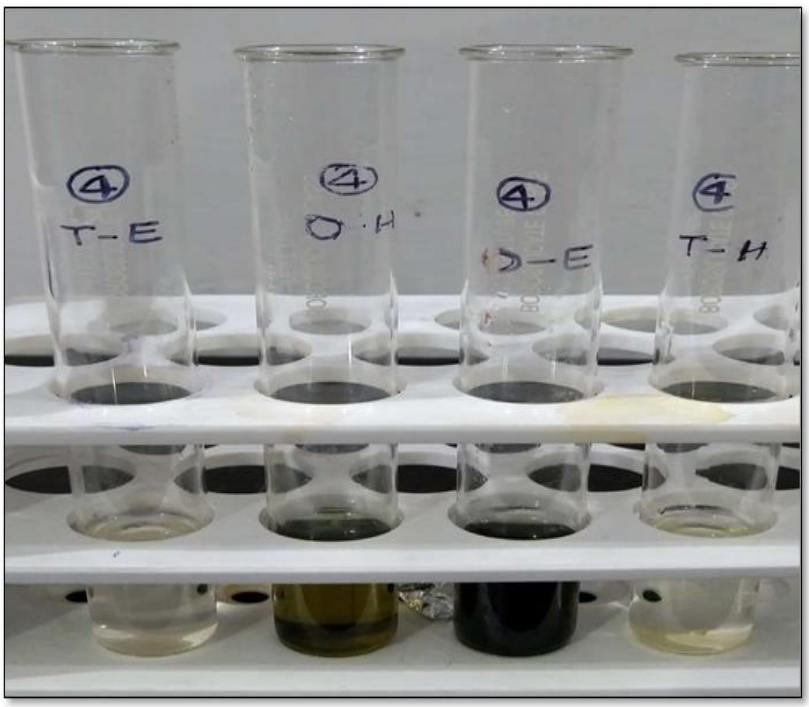


Fig. 8. Test for terpenoids (Salkowski's test):

(f). Alkaloids: Ocimum Sanctum (Ethanol & Hexane) produces an orange-reddish brown colour, while T. Cordifolia produces no colour.

Fig. 9. Test for Alkaloids (Dragendorff's test)

(g). Carbohydrates: Ocimum sanctum two test tube Ethanol & Hexane produces reddish brown precipitate and Tinospora Cordifolia test tubes produces no precipitate.

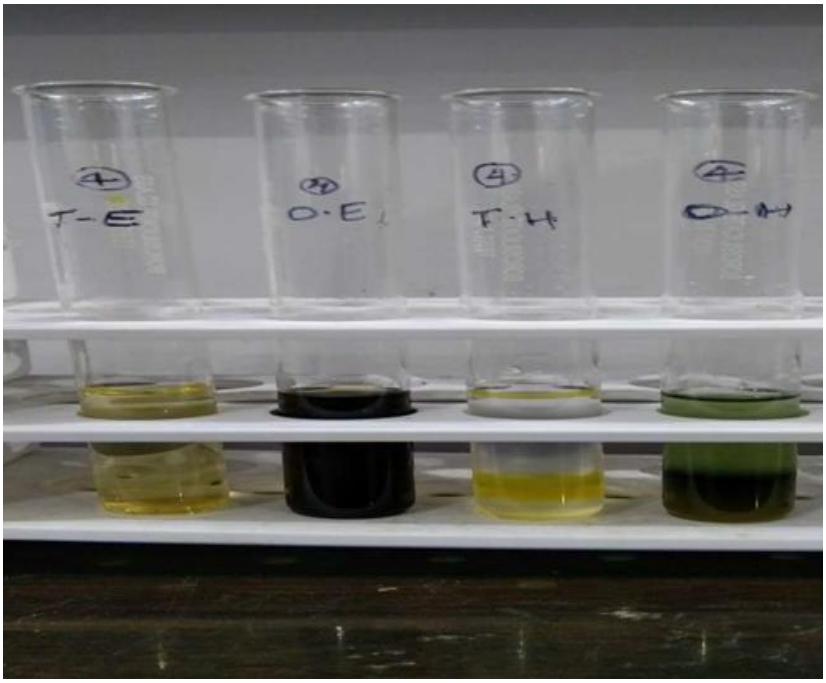


Fig. 10. Test for carbohydrates (Benedict's test)

(h). Cardiac glycosides: In test tubes of Ocimum sanctum (Ethanol & Hexane) gives good emergence of violet and brownish rings below the interface as compare to the Tinospora Cordifolia.

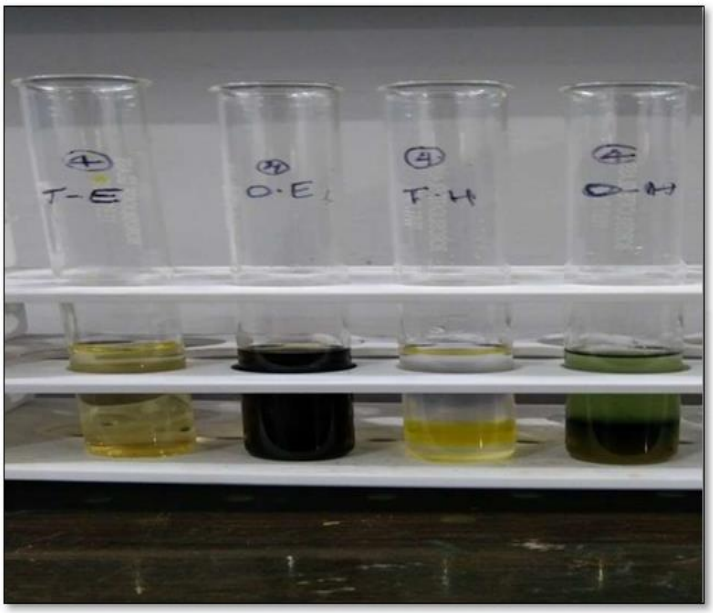


Fig. 11. Test for cardiac glycosides

(i). Tannis: The two test tubes of Ocimum Sanctum (Ethanol & Hexane) produces a blue-black coloration indicated the presence of tannins. And T. Cordifolia produces no color.

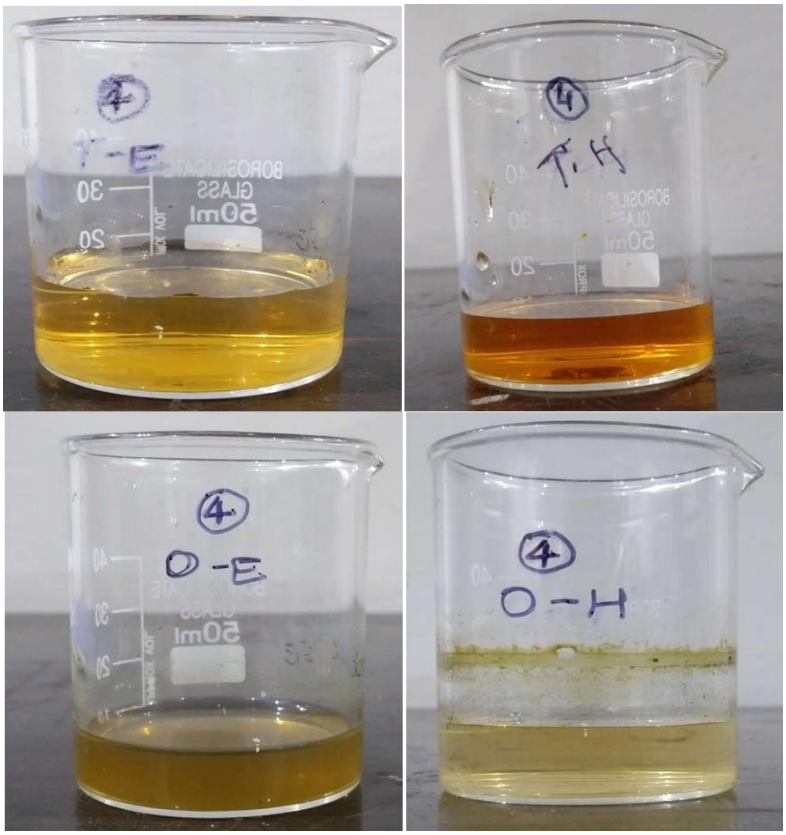


Fig.12. Test for tannins

(j). Steroids: In all test tubes a blue-green coloration indicated the presence of steroids

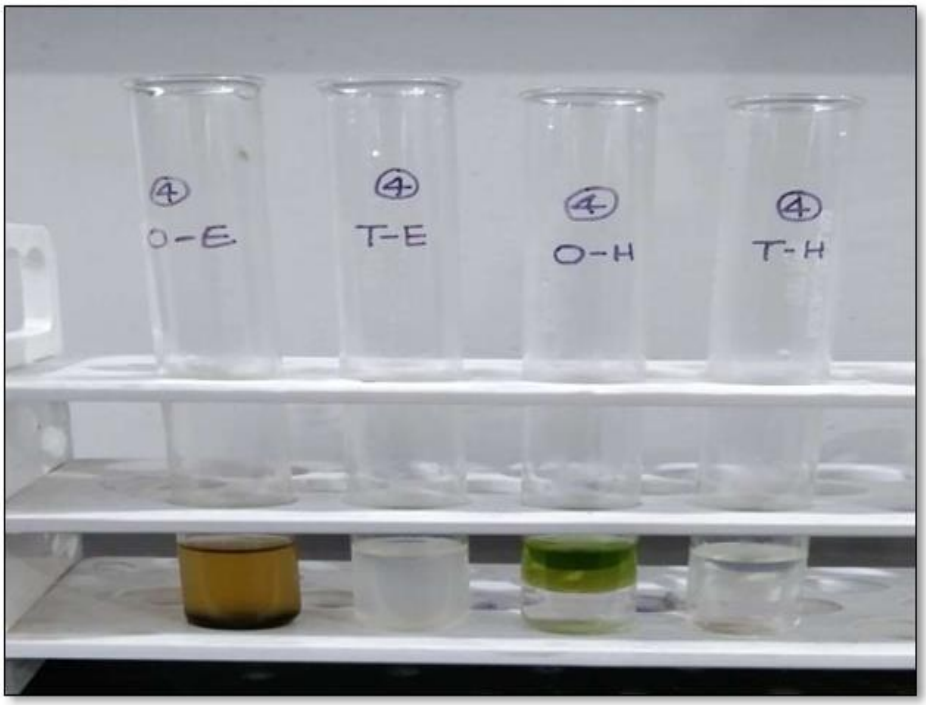


Fig. 13. Test for steroids (Lieberman-Burchard test)

Table 1. Result of Present and Absent of Bioactive Compound

Phytochemicals	O. Sanctum+ Ethanol	O. Sanctum+ Hexane	T.Cordifolia+Ethanol	T.Cordifolia+Hexane
Flavonoids	+	+	-	-
Saponins	+	+	+	+
Tannins	+	+	-	-
Steroids	+	+	+	+
Alkaloids	+	+	-	-
Cardiac Glycosides	+	+	+	+
Phenolics	+	+	+	+
Carbohydrate	+	+	-	-
Terpenoids	+	+	-	-
AA & Protein	+	+	-	-

4. FUTURE PROSPECTS

1. **Drug Discovery and Development:** The exploration of bioactive compounds in medicinal plants could lead to the discovery of novel drugs or therapeutic agents. As traditional medicine gains recognition and acceptance globally, there is a growing interest in identifying natural compounds with pharmacological activities [58].
2. **Alternative Medicine and Traditional Practices:** Research on medicinal plants aligns with the growing interest in alternative medicine and traditional healing practices. Understanding the bioactive compounds present in plants like Ocimum sanctum and Tinospora cordifolia could validate their traditional uses and provide scientific evidence for their efficacy [59].
3. **Pharmacological Applications:** The bioactive compounds identified through such studies may have various

pharmacological applications, including antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and immunomodulatory properties. These compounds could potentially serve as leads for the development of new drugs to combat various diseases [60].

4. **Nutraceuticals and Functional Foods:** Extracts or compounds derived from medicinal plants can be used in the formulation of nutraceuticals or functional foods with health-promoting properties [61].
5. **Bioprospecting and Sustainable Resource Management:** Investigating the bioactive compounds in medicinal plants contributes to bioprospecting efforts aimed at identifying valuable natural resources. Sustainable harvesting and cultivation practices can be implemented to ensure the long-term availability of these plant species while preserving biodiversity [62].
6. **Phytochemical Analysis Techniques:** Utilizing techniques like Soxhlet extraction for characterizing bioactive compounds provides valuable insights into the chemical composition of medicinal plants. This understanding is crucial for the standardization, quality control, and reproducibility of herbal products [63].
7. **Collaborative Interdisciplinary Research:** Studies on medicinal plants often involve interdisciplinary collaboration between botanists, pharmacologists, chemists, biochemists, and other specialists [64].
8. **Public Health Impact:** The development of new drugs or therapeutic agents from medicinal plants could have a significant impact on public health, particularly in regions where access to modern healthcare is limited. Affordable and accessible natural remedies could help address healthcare disparities and improve health outcomes [65].

5. CONCLUSION

In the present study*, Ocimum sanctum & Tinospora Cordifolia* is a medicinal plant having various types of compounds. The different bioactive compounds, including alkaloids, steroids, glycosides, tannins, etc have been discussed [66]. The present review spotlights the study & presence of Bioactive compounds. It has been used successfully in Ayurvedic medicine from the ancient era, and its products are used for their better economic and therapeutic utilization [67]. In this regard, further studies need to be carried out to explore *T. Cordifolia & Ocimum sanctum*. The crude drug (stem & leaf part) was successfully extracted by Soxhlet assembly using various solvents. Preliminary phytochemicals analysis of different extracts was carried out Using several colours and precipitate chemical reagents as per the described method & we got positive results which are shown in the given Table 1.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

INFORMED CONSENT

Using websites, review articles, and other sources to produce research content.

ETHICAL STATEMENT

A pharmacist ought to act with integrity and sincerity. A pharmacist abstains from behaviours that could undermine their commitment to acting in their patient's best interests, such as prejudiced acts or behaviours and unfavourable working environments that impair their judgment. A pharmacist upholds their reputation in the industry.

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CONFLICT OF INTEREST

The authors attest that they are free of any known financial or personal conflicts of interest that would taint the findings of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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