



Comparative study of the leaf extracts from the plant *thevetia peruviana* and *ocimum tenuiflorum*

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Abstract

From ancient time our forefather use different medicinal plant to treat different types of disease. Because different plants have medicinal activity such as antidiabetic, antibacterial, antifungal activity. In this study we use two different medicinal plant i.e *T. Peruviana* and *O. Tenuiflorum* and compare the phytochemical, antibacterial, antifungal and antioxidant activity of the leaf extracts from both the plant against *Staphylococcus aureus* and *Candida auris* The extracts of both the plants were prepared using soxhlet apparatus for ethanol extract and petroleum ether extract. The phytochemical activity of both the extracts were evaluated and the reported phytochemicals were alkaloids, flavonoids, tannins, terpenoids, saponins, phytosterols, proteins and phenolic compounds. The antimicrobial activities of both the extracts were evaluated using the disc diffusion method; the inhibitory zones were recorded in millimeters. The antioxidant activity of leaf extracts are assessed by means of DPPH free radical scavenging method. The ethanol extracts of *T. peruvianashows* a weaker Antifungal activity against *Candida auris* in comparison to *Ocimumtenuiflorum* In the Petroleum ether extract of both the plants, *T. peruvianashows* a stronger Antifungal activity against *Candida auris* in comparison to *Ocimumtenuiflorum* In the Ethanolic extract of both the plants *T.peruvianashows* a weaker Antibacterial activity against *Staphylococcus aureus*in comparison to *Ocimumtenuiflorum* As seen in the Petroleum ether extract of both the plants *Ocimumtenuiflorum* shows a weaker antibacterial activity against *Staphylococcus aureus* in comparison to *T.peruviana*.

Keywords: phytochemical, antibacterial alkaloid *Candida auris*

Introduction

Plants have been used for medicinal practices and purposes from the ancient period of time. It has been very beneficial for use to human. In the total population of world, 70-80% of human population depend on plants for health care. The use medicinal plants are considered to be safe due to their minimal or zero side effects. Recently WHO World's Health Organization) estimated that 80% of people worldwide rely on herbal medicines for some aspects of their primary health care needs. According to WHO, around 21000 plant species have the potential for being used as medicinal plants^[1].

As a group medicinal plants comprise approximately 8000 species and around 50% of all the higher flowering plant species in India. Many of the practitioners of the Indian system of medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative application^[2]. It is estimated that there are 7800 manufacturing units in India. It has been seen that there is a rising interest of herbal drugs in the health sector. The beneficial reason is that they might offer a natural safeguard against the development of certain conditions and be a reputed treatment for some disease or ailments. The Herbal preparations are considered moderate in efficacy and less toxic than the most commonly used pharmaceutical drugs. India has been identified as one of the top 12 mega-biodiversity centres of the world. This is because India has a vast area with wide variety of climate, soil, altitude and latitude. Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs.^[3] Most of the phytochemicals from plant sources such as phenolic and flavonoids have been reported to have positive effect on health and cancer

prevention. The study of medicinal plant starts with pre-extraction and extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. Traditional method such as maceration and soxhlet extractions are commonly used at the small research settings or at small manufacturing enterprise (SME) level^[3]. Significance advances have been made in the processing of medicinal plants such as the modern extraction methods, microwave assisted MAE), ultra-sound assisted UAE) and super-critical extraction SFE) in which these advances are aimed to increase yield at lower cost^[3]. Also the modifications on the methods are been continuously developed.

Apart from medicinal value, there are several plants which are used in food or food making process, perfume, pest control, natural dye, tea, firewood and so on. Plants play a great role in the human culture development all over the world or by maintaining the environment. In today's world, many plants are used and considered as home remedy in different parts of the world. After knowing the value of the medicinal plants, a lot of people started gardening them at their home.

Over the years, use of medicinal plants or herbal medicine is enormously increasing as the medicinal plants are important sources of pharmaceutical manufacturing. For the intervention of common ailments such as chicken pox, bronchitis, diarrhoea, common cold, fever, tonsillitis, nausea, vomiting, constipation, piles, hypertension, menstrual disorders there are so many remedies are given by the traditional medicine practitioners.

As we have seen our family member applying many natural and herbal method which is effective and beneficial for treatment of many diseases and to banish insects as well as

pest. Natural product such as neem, aloe-vera, cascabelaetc are crucial and beneficial and amongst them *T. peruviana* and *O. tenuiflorum* is much more important for treating some diseases. The species can be found easily and cost efficient.

Thevetia peruviana

Thevetia peruviana (*Cascabela thevetia*) is a poisonous plant which is native to Central and South America especially Mexico, West Indies, Brazil but frequently cultivated throughout the tropical including India and Sri Lanka as an ornamental plant. It is commonly known as Yellow Oleander or suicide tree or lucky nut. It is a evergreen tropical shrub. The leaves of this plant are arranged linearly, and glossy green in colour the stem of this plant is little greyish green in colour. It blooms flowers also in the summer season. The flowers are long funnel shaped with spirally twisted. The seeds are green in colour and become black after ripening. All parts of the plants contain milky juice and it is toxic in nature. All parts of the plants contain latex which is toxic. It is highly temperature tolerant and drought resistant in different parts of India.

The plant possesses many pharmacologically active compounds like, Alkaloids, glycosides, saponins, flavonoids, tannins and phenolic compounds and has anti HIV, anti-inflammatory, anti-spermatogenic, antitermite, antifungal, antidiarrheal, cytotoxic activity. The useful medicinal activities can form the base for the development of herbal medicines.

All parts of *T. peruviana* have medicinal value. The milky juice contains a compound called 'thevetin' which is used as a heart stimulating agent and other components are cardenolides called as Thevetin A and Thevetin B, peruvoside, nerrifolin, thvetoxin and ruvoside. By drying or by heating, these cardenolides are not demolished. The oil extracted from the seed is used as a biological pest control.

Vernacula Names

In addition to scientific name *T. peruviana* also have multiple common name that is shown below

Table 1: Vernacular names of *T. peruviana*

Language	Vernacular name
English	Yellow oleander lucky nut
Hindi	Peelikaner
Assamese	Karabi
Manipuri	Utonglei
Bengali	Kolkaphul

Taxonomical Classification

According to the botanical scheme, the plant is classified as follows:

Kingdom-Plantae
Class-Magnoliopsida
Order-Gentianales
Family-Apocynaceae
Genus-Cascabela
Species-*C. peruviana*

Ocimum tenuiflorum

From the ancient time, Ayurveda has already using *Ocimum tenuiflorum* (tulsi) for its great medicinal values. It is commonly called as Queen of herbs and Holy basil. The leaves of this plant are oval with a sharp tip. It is native to

the Indian subcontinent Southeast Asian tropics. There are eighteen types of tulsi are found worldwide and some of them which are commonly found in India are- Rama tulsi, Kishnatulsi, Amrita tulsi, Vanatulsi, Sweet basil, thai basil, purple basil, lemon basil etc. Holy basil is constructed with many branched subshrub, nearly 30-60 cm in length with hairy stems. The colour of leaves varies from green to purple. The leaves are 4-5 cm in size and petioled with a slightly toothed margin. The leaves of tulsi plants are strongly scented the plant blooms flowers also which are purplish in colour.

Traditionally tulsi is used as an ingredient of herbal tea or it is taken as fresh leaf, dried powder. Ayurveda is using tulsi for medicinal practices as a treating agent for many diseases. The extracts from the tulsi leaves are commonly used common cold, headache, heart diseases, abdominal disorders, inflammation etc. Over the years, tulsi is been used in the cosmetic industries as an ingredient of soap, shampoo, hair oil etc. Tulsi leaves are also used to deport insects by mixing with stored grains for many years. It is also cultivated for its essential oil. Tulsi is widely used for its sanctity and religious purposes.

There are some phytochemical constituents that are present in tulsi are- ursolic acid, rosmarinic acid, oleanolic acid, carvacrol, eugenol, beta-caryophyllene, linalool etc. As nutritional value, it contains vitamin A and vitamin C, minerals like iron, calcium, zinc, and other phytonutrients. The essential oil of tulsi contains mostly eugenol (~70%), beta-elemene (~11.0%), beta-caryophyllene (~8%) and germacrene (~2%) with various trace compounds, mostly terpenes.

Vernacular Names

In addition to scientific names, *Ocimum tenuiflorum* also have multiple common or local names which are shown below.

Table 2: Vernacular names of *O. tenuiflorum*

Language	Vernacular name
English	Holy basil
Hindi Bengali Gujrati	Tulsi
Assamese Telegu	Tulasi
Marathi	Tulasa
Malayalam	Trittavu
Tamil	Thulasi

Taxonomical Classification

According to botanical scheme, *O. tenuiflorum* is classified as follows

Kingdom-Plantae
Class-Angiosperms
Order-Lamiales
Family-Lamiaceae
Genus-*Ocimum*
Species-*O. tenuiflorum*

Methodology

Plant Sample Collection

The plant sample was collected in February 2019 from the various parts of Guwahati, Assam. Leaf were used for obtaining the extract for the experiment.

Extraction of plant material plant samples were washed with water and air dried at room temperature for 7 days. The dried leaves were powered using a mixer grinder and stored

at a tight container for future use. Two different solvents such as ethanol and petroleum ether were used for extraction.



Fig 1: Plant sample of *Thevetia peruviana* A1 Fresh leaves, A2 Dried leaves, A3 Leaves powder)

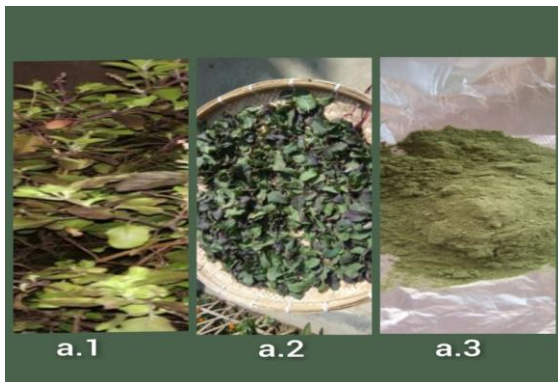


Fig 2: Plant sample of *Ocimum tenuiflorum* a1, Fresh leaves a2 Dried leaves, a3. Leaves powder)

Leaf Extract from leaves of *Thevetia peruviana* and *Ocimum tenuiflorum*-

Ethanol and Petroleum Ether preparation

50grams of the sieved powder was weighed and subjected to extraction in a soxhlet apparatus at room temperature and had been extracted in ethanol and petroleum ether. After extraction it had been evaporate to become dry in a porcelain dish and then it stored for phytochemical analysis.

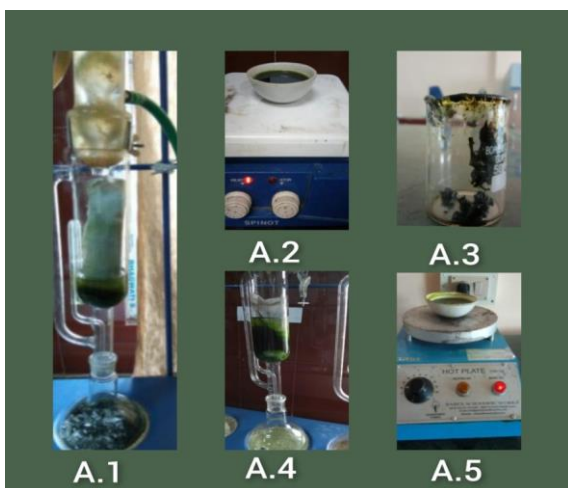


Fig 3: A1 & A4. Soxhlet Apparatus, A2. Evaporated plant extract, A3. Ethanol extract, A5. Petroleum ether extract

Qualitative Phytochemical Analysis-

The extracts in all the 2 solvents of leaves were tested for the presence of biological compounds by using following standard methods.

Test for Carbohydrates

Fehling's test for- Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to leaf extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presences of reducing sugars.

Benedict's test- Leaf extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presences of carbohydrate.

Molish's reagent- Aqueous or alcoholic solution of substance and 10% alcoholic solution on of substance and 10% alcoholic solution on of a α -naphthol, shake well then added conc. Sulphuric acid along with the side of the tube. A violet ring at the junction of two liquid confirmed the presences of carbohydrate.

Test for Phenols and tannins

2 drops of 5% ferric chloride was added in 2ml of extract, brown colour or dark green colour indicates the presence of tannins and phenol.

Test for Flavonoid

2ml of dilute sulphuric hydroxide was added to 2ml of extract. The appearance of yellow colour indicates the presences of flavonoid.

Test for Saponins

5ml of aqueous extract were mixed with 5ml of distilled water in test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presences of saponin.

Test for Glycosides and Steroids

Liebermann's test- Extracts were mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated sulphuric acid was added. A colour change from violet to blue to green indicated the presences of steroid nucleus, i.e, glyconeportion of glycoside.

Salkowski's test – Extracts were mixed with 2ml of choloform. Then 2ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presences of steroid ring, i.e, glycone portion of the glycoside.

Test for Alkaloids

Wagner's reagent- 2ml of extract was taken in a test tube. Acidified the solution by adding 0.1ml of HCl acid then added 0.1ml Wagner's reagent. Reddish brown colour precipitate was formed indicating the presence of alkaloids.

Mayer's reagent test- 1.2ml of extract was taken in a test tube. 0.2ml of dilute HCl was added then 0.1ml of Mayer's reagent was added formation of yellowish buff colour precipitate indicates the presences of alkaloids.

Test for Protein

The extracts were treated with few drops of concentrated Nitric acid solution. Formation of yellow colour indicates the presences of protein.

Collection of Test Organisms

The test organisms *Candida auris* and *S.aureus* were collected from the hospital laboratory itself.

Antimicrobial Assay

Media Preparation

Bacterial media Muller Hinton Media) and Fungal media Sabouraud dextrose agar)

36g of Muller Hinton Media (Hi-Media) and 36g of sabouraud dextrose agar media was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into Petri dishes. The plates with disc were used for the antimicrobial studies.

Disc-Diffusion Method

Antibacterial activities of the plant extract were tested using disc diffusion method. The prepared culture plates were inoculated with *Candida* strains and *S.aureus*.of microorganisms using spread plate method. Discs were made on the agar surface with cork borer. The extracts were poured into the disc using micro-pipettes. The plates were incubated at $37^{\circ}\text{C}\pm 2^{\circ}\text{C}$ for 24 hours for bacterial activity. The plates were observed for the zone clearance around the disc.

Results

Table 3: Phytochemical Test Results

Sample with Solvent	Test Name	Test	Observation	Inference
1) <i>Thevetia peruviana</i> with ethanol	Flavanoids	Extraction treated with few drops of lead acetate solution	Formation on yellow colour precipitate	Positive
2) <i>Ocimum tenuiflorum</i> with ethanol-				
3) <i>Thevetia peruviana</i> with petroleum ether				
4) <i>Ocimum tenuiflorum</i> with petroleum ether				
5) <i>Thevetia peruviana</i> with ethanol	Alkaloids	Filtrates were treated with few drops of Hager's reagent saturated solution of picric solution)	Formation on yellow colour precipitate	Positive
6) <i>Ocimum tenuiflorum</i> with ethanol-				Negative
7) <i>Thevetia peruviana</i> with petroleum ether				
8) <i>Ocimum tenuiflorum</i> with petroleum ether				
9) <i>Thevetia peruviana</i> with ethanol	Tannins	10 ml of bromine water and 0.5g aqueous extract	Decolourisation of bromine water	Positive
10) <i>Ocimum tenuiflorum</i> with ethanol-				Negative
11) <i>Thevetia peruviana</i> with petroleum ether				
12) <i>Ocimum tenuiflorum</i> with petroleum ether				
13) <i>Thevetia peruviana</i> with ethanol	Saponin	Small amount of extract was shaken with little quantity of water	Foam produced and persisting for 10mintues	Positive
14) <i>Ocimum tenuiflorum</i> with ethanol-				Negative
15) <i>Thevetia peruviana</i> with petroleum ether				
16) <i>Ocimum tenuiflorum</i> with petroleum ether				
17) <i>Thevetia peruviana</i> with ethanol	Phytosterol	Extract treated with chloroform and filtered. The filtrate treated with conc. Sulphuric acid after that shaken and allow to stand	No appearance of golden yellow colour	Positive
18) <i>Ocimum tenuiflorum</i> with ethanol-				Negative
19) <i>Thevetia peruviana</i> with petroleum ether				
20) <i>Ocimum tenuiflorum</i> with petroleum ether				
21) <i>Thevetia peruviana</i> with ethanol	Terpenoids	2.0ml of chloroform was added with 5 ml aqueous plant extract and evaporated on the water bath and then boiled with 3 ml of conc. Sulphuric acid	Grey colour formed	Positive
22) <i>Ocimum tenuiflorum</i> with ethanol-				Negative
23) <i>Thevetia peruviana</i> with petroleum ether				
24) <i>Ocimum tenuiflorum</i> with petroleum ether				
25) <i>Thevetia peruviana</i> with ethanol	Protein	Extract treated with few drops of nitric acid	Formation of yellow colour	Positive
26) <i>Ocimum tenuiflorum</i> with ethanol-				
27) <i>Thevetia peruviana</i> with petroleum ether				
28) <i>Ocimum tenuiflorum</i> with petroleum ether				

Antimicrobial Assay

The dried plant extracts of *Thevetia peruviana* was dissolved in 10% DMSO to get a concentration of 250 μ /ml distilled water and sterilized by filtration by μ m Millipore filters. Antimicrobial tests were then carried out by agar

diffusion method. Bacteria were cultured overnight at 37°C for 72 hour used as inoculums. Nutrient agar (20ml) was dispensed into sterile universal bottles. These were then inoculated, mixed gently and poured into sterile petri dishes. After setting a number 1-cup borer (6mm) diameter was

properly sterilized by flaming and used to make a uniform well in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The wells were filled with 50µl of the extract concentration of 100µg/ml and allow for diffuse 45 minutes). The plates were incubated at 37°C for 24hours for bacteria. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicate. The extract/fractions that showed antimicrobial activity.

Antibacterial Activity

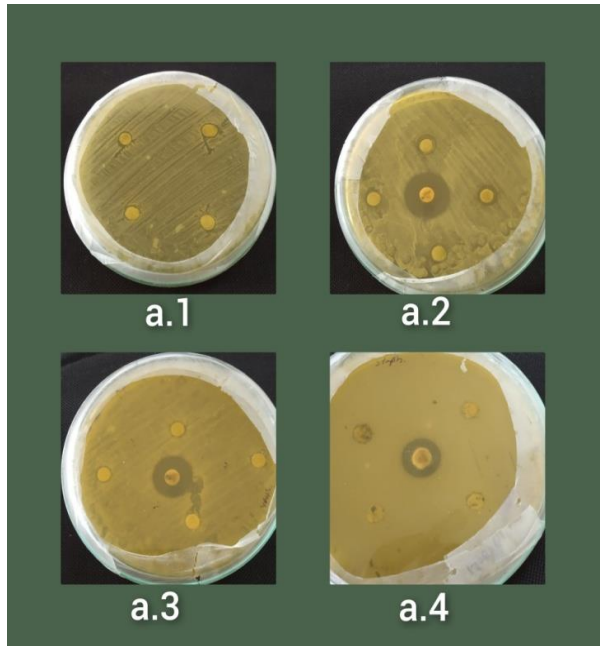


Fig 4: a1. *Thevetia peruviana* with ethanol against *Staphylococcus aureus* a2. *Ocimum tenuiflorum* with ethanol against *Staphylococcus aureus* a3. *Thevetia peruviana* with petroleum ether against *Staphylococcus aureus* a4. *Ocimum tenuiflorum* with petroleum ether against *Staphylococcus aureus*

Antifungal Activity

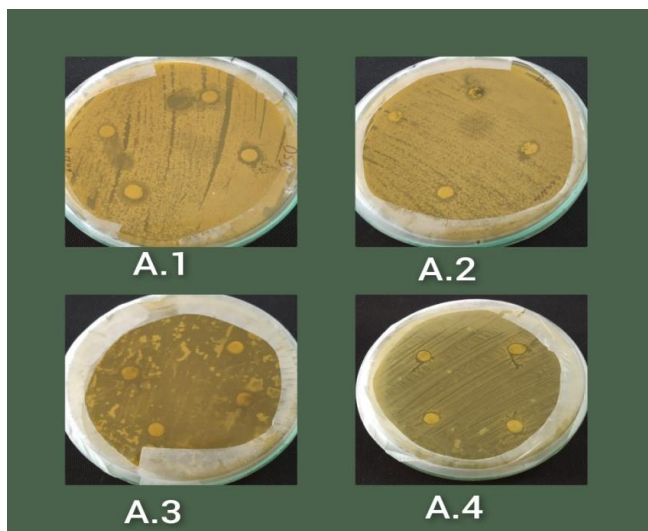


Fig 5: A1. *Thevetia peruviana* with petroleum ether against *Candida auris* A2. *Ocimum tenuiflorum* with petroleum ether against *Candida auris* A3. *Ocimum tenuiflorum* with ethanol against *Candida auris* A4. *Thevetia peruviana* with ethanol against *Candida auris*

Antibacterial Assay

Antibacterial assay of Tulsi and *T.peruviana* Ethanolic extract against *Staphylococcus aureus*

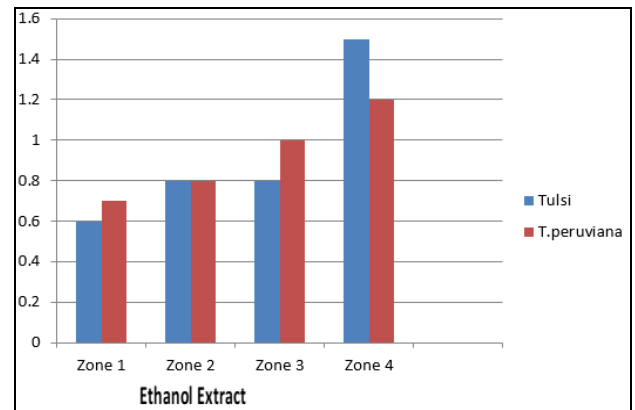


Fig 6: X-Axis – No.of zones Y-Axis – Zone of Inhibition against *Staphylococcus aureus*.

Antibacterial Assay of Tulsi and *T.peruviana* Petroleum Ether Extract against

Staphylococcus aureus.

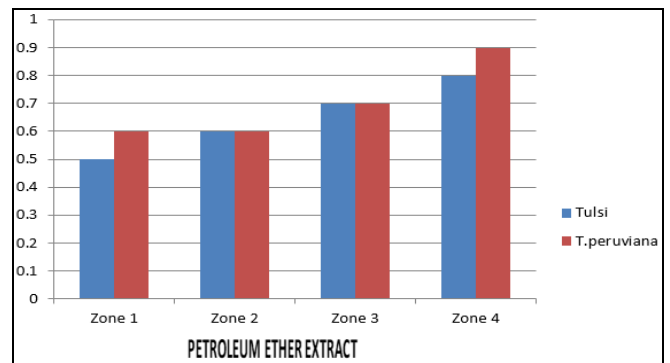


Fig 7: X-Axis – No. of Zones Y – Axis – Zone of Inhibition against *Staphylococcus aureus*.

Anti Fungal Assay

The antifungal activity of Tulsi and *T. peruviana* Ethanolic extract against *Candida auris*

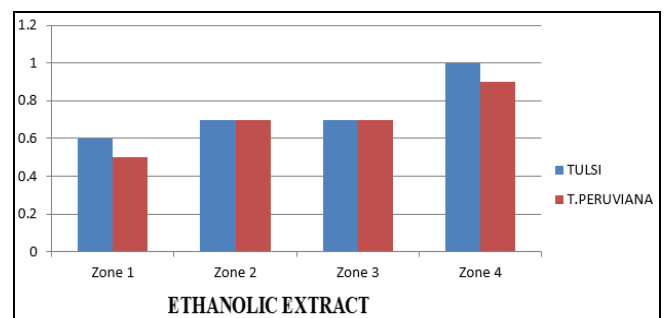


Fig 8: X-Axis–No. of Zones Y-Axis–Zone of Inhibition against *Candida auris*

The antifungal activity of Tulsi and *T.peruviana* Petroleum ether extract against *Candida auris*.

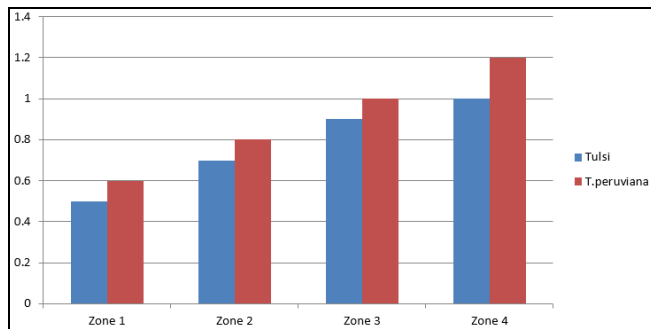


Fig 9: X-Axis–No. of Zones Y-Axis–Zone of Inhibition Against *Candida auris*

Comparative Study

A comparative study is a study in which a participant is randomly assigned to one of two or more different treatment groups for the purpose of comparing the effects of the treatment. Based on the observations of the different tests demonstrated upon the leaf extracts of the plant-*Thevetia peruviana* and *Ocimum tenuiflorum* a comparative study is been performed.

Phytochemical Analysis

The Phytochemical analysis of both the plants *Thevetia peruviana* and *Ocimum tenuiflorum* extract extracted using two different solvents Ethanol and Petroleum Ether was performed.

And based upon the observation of the different tests performed, it is analysed that the extracts of both the plants prepared using Ethanol as the solvent showed an almost equal number of positive results for the presence of the phytochemicals – Flavonoids Alkaloids Tannins Cardiac Glycosides Saponins Phytosterol test Terpenoids Protein Except for Phenols, as Phenols showed a negative result when tested on *Thevetia peruviana* and showed positive result when tested on *Ocimum tenuiflorum*.

Now, the analysis of the of the extract prepared of both the plants using Petroleum ether as the solvent gave a variant observation. The Tulsi extract showed most of the positive result for the presence of the phytochemicals while *Thevetia peruviana* showed a lesser amount of positive result for the presence of the Phytochemicals.

Anti Bacterial assay

The Anti-bacterial activity of the plants – *Thevetia peruviana* and *Ocimum tenuiflorum* was measured by using the Disk diffusion method. The zone of inhibition of both the plant extract prepared from two different solvents Ethanol and Petroleum ether) was observed against the bacteria *Staphylococcus aureus*.

In case of the plant extracts prepared using Ethanol, the zone of inhibition measured revealed that Tulsi *Ocimum tenuiflorum* showed a greater zone of inhibition as compared to *Thevetia peruviana* Thus proving that Tulsi extract prepared from ethanol has greater antibacterial property than Yellow Oleander extract.

In case of the plant extracts prepared using Petroleum Ether, the zone of inhibition measured revealed that yellow oleander *Thevetia peruviana*) showed a more clear zone of inhibition as compared to Tulsi *Ocimum tenuiflorum*). Thus proving that Yellow Oleander plant extract prepared using Petroleum ether has greater antibacterial activity than Tulsi.

Anti Fungal assay

The Anti fungal activity of the plants – *Thevetia peruviana* and *Ocimum tenuiflorum* was measured using Disk diffusion method. The zone of inhibition of both the plant extract prepared from two different solvents Ethanol and Petroleum Ether) was observed against the Fungus *Candida auris*.

In case of the plant extracts prepared using Ethanol, the zone of inhibition measured revealed that Yellow Oleander *Thevetia peruviana*) showed a better and clear zone of inhibition in comparison to Tulsi *Ocimum tenuiflorum*). Thus proving that Yellow Oleander plant extract prepared using Ethanol has greater Antifungal potential as compared to Tulsi.

In case of the plant extract prepared using Petroleum Ether, the zone of inhibition measured revealed that Yellow Oleander *Thevetia peruviana*) showed a better and clear zone of inhibition in comparison to Tulsi *Ocimum tenuiflorum* Thus proving that Yellow Oleander plant extract prepared using Petroleum Ether has greater Antifungal potential as compared to Tulsi.

Conclusion

We conclude that *Thevetia peruviana* leaf extract with petroleum ether against *Staphylococcus aureus* as the potential and has more medically active compounds and in more quantity due to which it can be replaced with *Ocimum tenuiflorum* by herbal industries for producing herbal medicines. Due to this, the general people will be able to have a greater access to herbal medicines in terms of quality and quantity. This will also lead to the increase in production of the Herbal medicinal Industries which will be beneficial both in terms of commercial value and to the mankind.

By the analysis of the of the extract, The Tulsi extract showed most of the positive result for the presence of the phytochemicals while *Thevetia peruviana* showed a lesser amount of positive result for the presence of the Phytochemicals. Antimicrobial activity was confirmed by the selected plant species and the results revealed that plant extracts varied in their efficacy for inhibiting the microbial growth of the tested pathogens.

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