



Phytochemical Analysis And Evaluation Of Polyherbal Cream For Wound Healing

Ingle Shruti Babarao*, Ingale Mukta Govind, Ingole Gajiram Balaji, Jadhav Aniket Amrut, Jadhav
Dhanashri Ram.

D.K. Patil Institute of Pharmacy, Sayal Road, Loha.

ABSTRACT

Wound healing is a dynamic and complex process that involves inflammation, proliferation, and tissue remodeling. Herbal formulations have gained increasing importance due to their therapeutic potential, safety, and reduced side effects. The present study focuses on the formulation, phytochemical analysis, and evaluation of a polyherbal cream incorporating extracts of *Ocimum sanctum* (Tulsi) and *Tinospora cordifolia* (Gudvel/Giloy), both well known for their antimicrobial, anti-inflammatory, and antioxidant properties.

Preliminary phytochemical screening of the plant extracts revealed the presence of bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, and phenolic compounds, which contribute significantly to wound healing activity. The polyherbal cream was formulated using a suitable base and evaluated for various physicochemical parameters including pH, viscosity, spreadability, homogeneity, and stability.

The wound healing efficacy of the prepared formulation was assessed through appropriate experimental models, showing enhanced wound contraction, faster epithelialization, and reduced healing time compared to standard and control groups. Additionally, the formulation exhibited notable antimicrobial activity, helping to prevent wound infections.

The study concludes that the polyherbal cream containing Tulsi and Gudvel demonstrates significant wound healing potential due to the synergistic action of its phytoconstituents. This formulation can serve as a promising, safe, and effective alternative for wound care management.

KEYWORDS: *Tinospora Cordifolia*, Pharmacological Activity, Active components, Nutritional Potential, Anti-inflammatory, Medicinal plant, Gulvel, Tulsi, Anti-oxidant.

INTRODUCTION

Wound

A wound is defined as any tissue injury that disrupts anatomical integrity and leads to functional loss. The ability of an organism to repair or regenerate tissues is a definite advantage for survival.

Wound Infection

A wound infection occurs when bacteria enters a break in the skin. The infection may involve just the skin, or affect deeper tissues or organs close to the wound.

Signs and Symptoms of a Wound Infection

Your symptoms may start a few days after you get the wound, or may not occur for a month or two after the wound happens:

1. Fever
2. Warm, red, painful, or swollen skin near the wound
3. Blood or pus coming from the wound
4. A foul odor coming from the wound

Risky factors of wound healing

1. Poorly controlled diabetes
2. Prior surgery
3. Radiation therapy or chemotherapy
4. Conditions associated with hypoxia and/or poor tissue perfusion (e.g., anemia, cardiac or respiratory disease, arterial or vascular disease, renal impairment, rheumatoid arthritis, shock)
5. Immune system disorders (e.g., acquired immune deficiency syndrome, malignancy)
6. Inappropriate antibiotic prophylaxis, particularly in acute wounds
7. Protein-energy malnutrition
8. Alcohol, smoking, and drug abuse

9. +/- erythema Lymphangitis Crepitus

10. Age ⁽¹⁾.

Anti-microbial agent

- Anti-microbial agent any of a large variety of chemical compound and physical agents that are used to destroy microorganisms or to prevent their development.
- Antimicrobial drugs can be synthetic chemicals, metabolic products from microorganisms, or chemical substances from plants. They can prevent or treat disease caused by bacteria, fungi, viruses, protozoa, or helminths.
- The name ‘antimicrobial’ is an umbrella term for anything that inhibits or kills microbes including antibiotics (which target bacteria), antifungals (which target fungi) and antivirals (which target viruses).

Examples of Antimicrobials

Both viruses and fungi grow in very different ways from bacteria and are composed of different materials. There are far fewer antiviral and antifungal drugs that have been approved for use.

- **Antivirals**

Viruses require a live cell to replicate inside and they are very specific, meaning that each one will only replicate inside a certain type of cell and there are lots of different ways in which this happens. Once inside the right cell, a virus is copied by the cellular machinery. This means that any drug that is developed to inhibit virus replication will be harmful to the cell’s own machinery, often making antiviral drugs more toxic than antibiotics. Some antivirals are very cleverly designed to only be activated once inside an infected cell.

- **Antifungals**

Antifungals have been developed to specifically interfere with targets that are unique to fungal processes. For example, azole treatments specifically prevent fungi from making important fungal cell membrane components.

- **Antiparasitic drugs**

Parasites like the protozoa that cause malaria often have complex life cycles, with different stages of their development happening in different host types e.g. mosquitoes and human blood or liver cells. Furthermore, parasites can live both inside and outside host cells making antiparasitic drugs very difficult to develop. So often they are targeted at key stages of parasite development (2).

Common Side Effects

Antimicrobial agents are commonly used to treat various infections and diseases, but they can also cause a range of side effects. It is important to be aware of these potential side effects in order to use these medications safely and effectively.

Some of the most common side effects of antimicrobial agents include:

- **Gastrointestinal Disturbances:** Many antimicrobial agents can disrupt the balance of normal flora in the gut, leading to symptoms such as diarrhea, nausea, and abdominal pain. According to the World Health Organization, gastrointestinal disturbances are reported in up to 20% of patients taking antimicrobial agents.
- **Allergic Reactions:** Some individuals may develop allergic reactions to antimicrobial agents, ranging from mild rashes to severe anaphylaxis. The Centers for Disease Control and Prevention reports that approximately 5-10% of patients experience some form of allergic reaction to antimicrobial agents.
- **Drug Resistance:** Prolonged or inappropriate use of antimicrobial agents can contribute to the development of drug-resistant bacteria. This can have serious implications for public health, as it limits the effectiveness of these medications in treating infections.
- **Organ Toxicity:** Certain antimicrobial agents, particularly those used to treat serious infections, can have toxic effects on the liver, kidneys, or other organs. According to the National Institutes of Health, organ toxicity is a rare but serious side effect of some antimicrobial agents (3).

It is important to note that the likelihood and severity of these side effects can vary depending on the specific antimicrobial agent being used, as well as individual patient factors such as age, underlying health conditions, and other medications being taken.

To minimize the risk of side effects, it is essential to use antimicrobial agents judiciously and according to the guidance of a healthcare professional. This includes completing the full course of treatment as prescribed, avoiding the use of antimicrobial agents for non-bacterial infections, and not sharing or using leftover antimicrobial agents from previous treatments.

Common types of antimicrobial agents include :-

- **Antibiotics** Antibiotics combat microbes, specifically killing or inhibiting bacteria.
- **Antifungal drugs** These selectively target fungal pathogens, with major groups including polyenes, azoles, and ally amines.
- **Antiparasitic drugs** These are divided into antiprotozoal agents and anthelmintic agents.

INTRODUCTION

Tinospora Cordifolia:-

- Commonly known Guduchi, Amrita, Gurach, and Tinospora.
- *Tinospora cordifolia* also called Amrita, Gila, Guduchis widely used in Ayurveda system of medicine (1).

- It is widely distributed in India, Malasia, Indonesia and Thailand.
- The Hindi name of plant is Gila.
- In India the common medicinal herb is *Tinospora Cordifolia*.
- It is frequently used to treat conditions wound Infections , Rheumatism , Diabetes, Skin conditions , Wound infections , Tooth infection , Jaundice , Hypertension and common cold (4).

Fig.1. *Tinospora Cordifolia*

Parts used to treat disease

- **Leaves :-**
Leaf powder and decoction is reported to help heal gout, ulcers, jaundice, fever, wounds, and blood sugar when combined with cow's milk.
- **Steam :-**
Stem starch, also known as sativa, is used as a tonic for fever, skin disorders, and jaundice. Stem extract can be and in The for a scorpion mixture of root
- **Barks :-**
In North combined with



administered both alone combination with honey. recommended remedy sting and snake bite is a and stem.

Gujarat, India, milk is the plant's stem and roots

to treat cancer. Fruits Both jaundice and rheumatism are treated with them.

- **Roots :-**

Aerial roots are thread-like, long, fill form, squairsh-shaped roots that grow downward from mature branches or clipped stem segments and occasionally reach the ground through continual growth(3).

A Gulvel (*Tinospora Cordifolia*) plant project typically revolve around utilizing the plant's medicinal properties for various health benefits, promoting sustainable cultivation, and potentially developing new pharmaceutical applications. Specific objectives might include studying its immunomodulatory, antioxidant, anti-inflammatory, and anti-diabetic properties, as well as exploring its potential in treating respiratory illnesses and even cancer. Furthermore, projects might focus on improving

- Cultivation techniques, developing value-added products, and raising awareness about its traditional uses and modern scientific validation (5).

- Here's a more detailed breakdown of potential objectives:

1. Medicinal Properties and Drug Development:

- Investigating Therapeutic Applications:

A primary objective is to thoroughly research and validate the medicinal properties of Gulvel, including its traditional uses and potential for treating various ailments.

- Developing Novel Therapies:

This includes exploring the plant's potential in creating new drugs or therapies for diseases like diabetes, cancer, and immune disorders, potentially offering safer and more effective alternatives to conventional treatments.

- Understanding Mechanisms of Action:

Researching the specific ways in which Gulvel's bioactive compounds interact with the body to produce desired therapeutic effects.

2. Cultivation and Sustainability:

- Optimizing Cultivation Practices:

Developing efficient and sustainable methods for cultivating Gulvel, including ideal soil conditions, irrigation, and pest management.

- Promoting Organic Cultivation:

Focusing on organic farming practices to ensure the production of high-quality, chemical-free medicinal plants.

- Developing Disease-Resistant Varieties:

Identifying and breeding strains of Gulvel that are more resistant to common diseases and pests, reducing the need for chemical interventions.

3. Value Addition and Economic Viability:

- Creating Value-Added Products:

Developing various product forms of Gulvel, such as tablets, capsules, extracts, and teas, to cater to diverse consumer needs.

- Promoting Sustainable Livelihoods:

Supporting local communities by providing them with opportunities for income generation through the cultivation and processing of Gulvel.

- Exploring Export Potential:

Identifying opportunities for exporting Gulvel and its value-added products to international markets (6).

- **Continued Exploration of Medicinal Properties:**

Further research is expected to delve deeper into Gulvel's medicinal properties, potentially leading to the development of new drugs and therapies.

- Standardized Formulations:

One challenge with Gulvel is the lack of standardized formulations, which can lead to variations in effectiveness. Future research may focus on creating consistent and reliable products.

- Increased Awareness and Acceptance:

As research continues to validate Gulvel's health benefits, it is likely to gain wider recognition and acceptance in mainstream healthcare systems, not just in traditional medicine.

- Potential for Disease Management:

Gulvel's potential in managing conditions like diabetes, liver disorders, and even certain types of cancer is being explored. Future research may lead to more effective and natural approaches to managing these diseases.

- Sustainable Cultivation:

With increasing demand for Gulvel, sustainable cultivation practices will become crucial to ensure its availability for future generations (7).

Ocimum sanctum:-

- Tulsi (*Ocimum sanctum*), commonly known as Holy Basil, holds a significant place in the Hindu religious tradition and Indian culture.
- The name 'Tulsi' signifies 'the incomparable one', while another of its names, Vishnupriya, refers to its divine association as 'the beloved of Lord Vishnu'.

- Tulsi is commonly grown in Indian households and is deeply rooted in the country's spiritual and medicinal heritage. Belonging to the family *Lamiaceae*,
- It exists in two main varieties-Rama (light) and Krishna (dark) Tulsi the latter being more commonly used in worship and known for its superior medicinal value.



Fig.2. *Ocimum sanctum*

Practical uses of Tulsi

Tulsi show numerous medical properties. It helps to treat cancers, ulcers, inflammations, liver problems and many more.

- **Anti-stress properties**

The vitamins like vitamin C and other antioxidants like camphene, repairs free radicals damage and minimizes the stress caused by these free radicals. It helps to calm blood pressure, nerves and inflammation thus reduces stress.

- **Acts against Respiratory disorders**

The components in essential oil of tulsi plant such as Camphene, Eugenol and Cineole, help to treat problems like asthma, cold and cough, influenza and other problems like bronchitis. In the problem of asthma it is very helpful since it relieves congestion and facilitates smoother breathing.

- **Against lung diseases**

The phytochemicals in tulsi such as Camphene, Eugenol, vitamin C and Cineole mediate their action against congestion, lung infections and shows healing of lung due to problems like tuberculosis, smoking and lung cancer.

- **Oral health**

Tulsi shows the activities of oral disinfectant and mouth freshener. The chemical components present in it kills approximately 99% of the mouth germs and bacteria that can cause dental cavities, tartar, bad breath and plaque. This is also effective against mouth ulcers.

- **Anti-microbial activities**

It is helpful in treating many of germs, bacteria, virus and fungal infections. Diseases like malaria, typhoid, flu, any kind of fungus, gonorrhea etc. can be cured by the effects of tulsi.

- **Anti-cancer properties**

Phytochemicals in tulsi leaves such as Eugenol and more other shows modulatory effect against carcinogen metabolic enzymes. Kidney problems Tulsi acts as a detoxifier and diuretic so helps to reduce uric acid level, urination levels and dissolution of kidney stones.

- **Headaches and fever**

Headaches due to migraines, cold and cough or high blood pressure can be cured by tulsi. Tulsi helps to cure that microbial infections and hence treats fever.

- **Heart problems**

Vitamin C and antioxidants like eugenol protects heart from free radicals and lowers the level of cholesterol.

- **Other functions**

Tulsi helps to treat various skin infections like dryness and pimples. Tulsi phytochemicals like vitamins and other nutrients protects body from free radicals, hazardous byproducts of cellular metabolism. So helps to regain health and avoid premature aging. It also soothes eyes, reduces inflammation and stress and gives relief from conjunctivitis, boils and many other problems of the eyes (8).



➤ **Used parts of Tulsi**

1. Tulsi Leaves
2. Tulsi Roots
3. Tulsi Seeds

Fig. Ocimum sanctum

PLANT PROFILE

GULVEL

- **Scientific Name:** - *Tinospora Cordifolia*
- **Biological source:** - It is made out developed, dried segments of '*Tinospora Cordifolia* miers' stem.
- **Family :-** *Menispermaceae*
- **Active ingredients:-**
Giloy (*Tinospora cordifolia*) contains many active chemical constituents, including Alkaloids, Glycosides, Steroids, Flavonoids, Phenols, Tannins, Terpenoids, Polysaccharides, Essential oils, and Fatty acids.
- It can be grow in nearly every climate the plant refers a warm one and is extremely stiff.
- Planting is usually done during rainy season (9).

USES:-

- Treat Diabetes
- Treat Asthma
- Treat Chronic Fever
- Boost Immunity
- Reduce Gouty Arthritis
- Treat Eye Disorder
- Anti-allergic
- Anti-diabetic



- Anti-microbial
- Anti-cancer
- Anti-depressant

TULSI

- **Scientific Name :** *Ocimum sanctum*, *Holy basil*
- **Biological source:** Tulsi consists of fresh and dried leaves of *Ocimum sanctum* Linn, belonging to family *lamiacea*.
- **Family:** *Lamiacea*
- **Active ingredients:**
This plant contains a wide range of active compounds, including carvacrol, β - elemene, β -caryophyllene, rosmarinic acid, ursolic acid, eugenol, and germacrene. These bioactive substances contribute to Tulsi's reputed diuretic and immune boosting properties (10).



USES:-

- Immuno-modulator
- Anti-oxidant
- Anti-bacterial
- Immunity Booster
- Stress and anxiety reduction
- Respiratory health
- Heart health
- Diabetes management
- Digestion and detoxification
- Skin and hair care
- Pain and fever relief

Selection of material

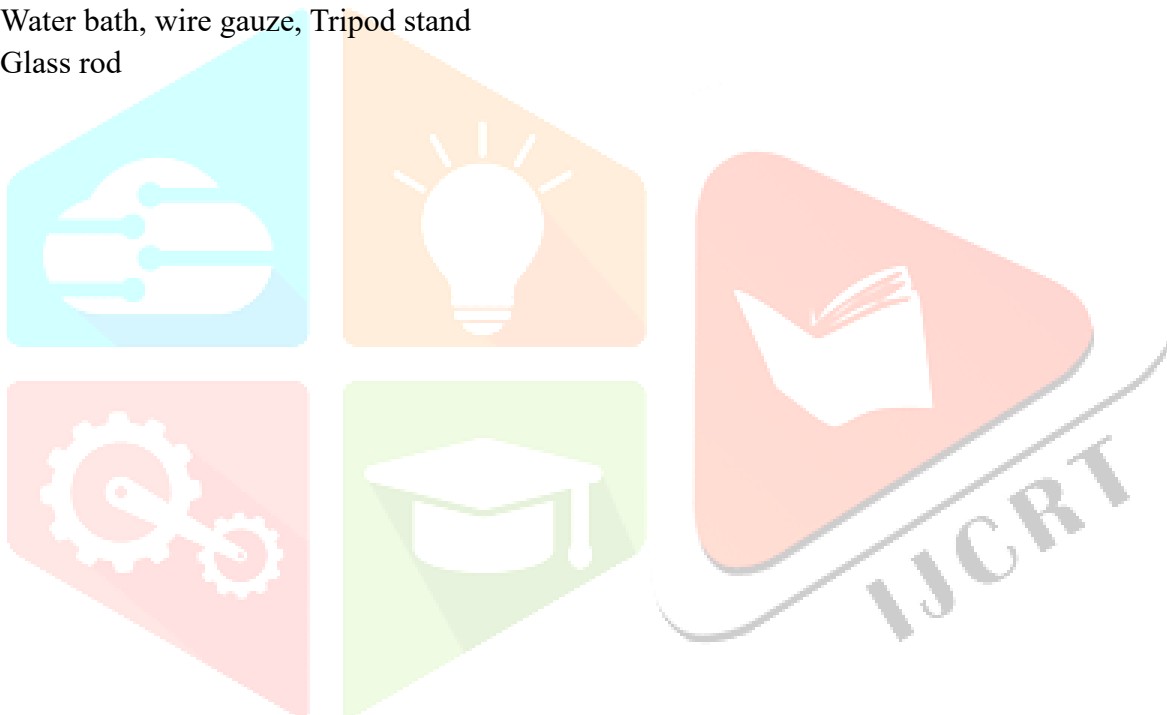
Material / Ingredient

1. Gulvel Sample
2. Tulsi sample
3. Excipient (Beeswax, Liquid paraffin, Borax, Methylparaben)

Collection of sample: from local market

Equipment:-

1. Weighing balance
2. Mortor pestor
3. Measuring cylinder
4. Beaker
5. Water bath, wire gauze, Tripod stand
6. Glass rod



Qualitative Analysis of *Tinospora Cordifolia* & *Ocimum sanctum*

Sr .no.	Chemicals	Procedure
1	Flavonoids	1) NaoH In this test added the high amount of NaoH standard solution in test extract of sample 2) sulphuric acid (H ₂ SO ₄) Test added of H ₂ SO ₄ in the extract
2	Phenolic compound and tannins	1) Ferric chloride (FeCl ₃) test in this test 2-3 ml of methanolic extract of sample and added ferric chloride (5%) solution 2) Gelatin test – in this test 2 -3 ml of methanolic exact and added gelatin solution 3) Lead acetate test - In this test 2-3 ml of methanolic extract and added acetic acid standard solution.
3	Carbohydrates	1) Fehling's test- Fehling (A and B) solutions were mixed (1 ml each) with each other than boiled for a minute. Then equal amount of extract of leaves was added 2) Molish's test- In this test 2-3 ml of leaves methanol extract was taken then added few (1-2) drops normal size of alpha-naphthol standard solution with alcohol, shaken and added the conc. H ₂ SO ₄ from one side of tube.
4	Proteins	1) Biuret test- In this test about 3 ml of leaves extract, then 4% sodium hydroxide standard solution and 1% standard copper sulphates solution was added. Alkaloid test 2) Ninhydrin test- In this test about 3 ml of metallic extract of leaves

		solution was heated then 2-3drops of 5% Ninhydrin standard solution was added then boiled for 10 minutes.
5	Alkaloid test	1) Mayer's test- In this test methanolic extract of leaves was evaporated then added dil. HCL standard, then shaken and filtered. Taken about 2-3 ml of filtered sample and added Mayer's reagent in few drops. 2) Dragendroff's test- - In this test methanolic extract of leaves was evaporated then added dil. HCL standard, then shaken and filtered. Taken about 2-3 ml of filtered sample and added Dragendroff reagent drop by drop.
6	Fats and oils	1) Oil globule test- In this test thick section of leaves extract was put on clear glass slide then added reagent which was Sudan Red in a drop, then slide was washed by using the alcohol (50%). After this this sample slide was deep in glycerin and observed under the microscope (16).

Quantitative Analysis of *Tinospora Cordifolia*

1) Total phenolic content (TP) determination

Folin–Ciocalteu reagent is main and standard chemical was used for the deciding the quantity of phenolic compounds in the dried sample. The extract approximately (50 mg) was combined with about 0.5 mL of Folin–Ciocalteu reagent and then mixed in distilled water (7.5 mL). After 10 minutes, 1.5 mL of 20 percent sodium carbonate (w/v) was poured. The solution was steamed in a water bath for about at 40 degrees Celsius for 20 minutes and chilled. A spectroscopy instrument was normally used to recording and determination of the absorbance at 755 nm. The calibration graph (curve) of Gallic acid was used to determine the quantity of TPC (Total Phenolic Content) in the concentration range in between 10-100 µg/ml. Gallic acid equivalents method (GAE) g/100 g were used to calculate the result.

2) Total flavonoid content (TF) determination

The extract sample in solvent methanol of umber plants (0.5 mL) was combined with 2.2 mL of filtered water as well as 5% NaNO₂ standard solution (0.15 mL). After 6 minutes, 0.3 mL of 10% AlCl₃6H₂O

solution was inserted and the prepared mixture allowed to stand for 5 minutes. Then 1 mL of 1M standard NaOH was added. After whirling the mixture, the absorbance was taken with a spectrophotometer at 510 nm. The extract's flavonols were converted to Gallic acid equivalents. The calibration curve Graph was madewith Gallic acid (standard solutions of 6.25, 12.5, 25.0, 50.0, 80.0, and 100.0 µg/ ml in 80 percent ethanol (V/V)).

3) Total tannin content (TT) determination

Folin-Ciocalteu reagent method is one of the important method was also used to determination of all tannin content (TF). The sample of gulvel extract (dried) taken about 0.1 ml and insert into a flat volumetric flask which is about 10 ml, then 7.5 ml of deionizedwater, after this 0.5 ml of standard Folin-Ciocalteu reagent which is important reagent to determine the TPC, then about 1 ml of standard sodium carbonates solution and diluted in 10 ml with (deionized) distilled water. The mixture of prepared sample was shaken properlyand kept in the room at normal temperature for about 30 min. One set which prepared for the reference standard (5-6 concentration) solutions of tannic acid (6.25, 12.5, 25.0, 50.0, 80.0, and 100.0 µg/ ml) this concentrations were prepared by the same type as described.

Quantitative Analysis of *Ocimum sanctum*

1) Total phenolic content (TP) determination

Folin–Ciocalteu reagent is main and standard chemical was used for the deciding the quantity of phenolic compounds in the dried sample. The extract approximately (50 mg) was combined with about 0.5 mL of Folin–Ciocalteu reagent and then mixed in distilled water (7.5 mL). After 10 minutes, 1.5 mL of 20 percent sodium carbonate (w/v) was poured. The solution was steamed in a water bath for about at 40 degrees Celsius for 20 minutes and chilled. A spectroscopy instrument was normally used to recording and determination of the absorbance at 755 nm. The calibration graph (curve) of Gallic acid was used to determine the quantity of TPC (Total Phenolic Content) in the concentration range in between 10-100 µg/ ml. Gallic acid equivalents method (GAE) g/100 g were used to calculate the result.

2) Total flavonoid content (TF) determination

The extract sample in solvent methanol of umber plants (0.5 mL) was combined with 2.2 mL of filtered water as well as 5% NaNO₂ standard solution (0.15 mL). After 6 minutes, 0.3 mL of 10% AlCl₃6H₂O solution was inserted and the prepared mixture allowed to stand for 5 minutes. Then 1 mL of 1M standard NaOH was added. After whirling the mixture, the absorbance was taken with a spectrophotometer at 510 nm. The extract's flavonols were converted to Gallic acid equivalents. The calibration curve Graph was madewith Gallic acid (standard solutions of 6.25, 12.5, 25.0, 50.0, 80.0, and 100.0 µg/ ml in 80 percent ethanol (V/V)).

3) Total tannin content (TT) determination

Folin-Ciocalteu reagent method is one of the important method was also used to determination of all tannin content (TF). The sample of gulvel extract (dried) taken about 0.1 ml and insert into a flat

volumetric flask which is about 10 ml, then 7.5 ml of deionized water, after this 0.5 ml of standard Folin-Ciocalteu reagent which is important reagent to determine the TPC, then about 1 ml of standard sodium carbonates solution and diluted in 10 ml with (deionized) distilled water. The mixture of prepared sample was shaken properly and kept in the room at normal temperature for about 30 min. One set which prepared for the reference standard (5-6 concentration) solutions of tannic acid (6.25, 12.5, 25.0, 50.0, 80.0, and 100.0 µg/ ml) this concentrations were prepared by the same type as described (18).

PREFORMULATION STUDY :-

1. Bulk density
2. Tapped density
3. Carr's index
4. Hausner's ratio
5. Angle of repose
6. % Ash value

Bulk density

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the inter-particulate void volume.

$$\text{FORMULA: BULK DENSITY} = \frac{\text{MASS}}{\text{BULK VOLUME}}$$

Tapped density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

$$\text{FORMULA: TAPPED DENSITY} = \frac{\text{MASS}}{\text{TAPPED VOLUME}}$$

Carr's index

Carr's Index of any solid is calculated for compressibility of a powder which is based on true density and bulk density.

$$\text{FORMULA: CARR'S INDEX} = \frac{\text{TAPPED DENSITY} - \text{BULK DENSITY}}{\text{TAPPED DENSITY}} \times 100$$

Hausner's ratio

Hausner ratio is defined as the ratio of a powder's tapped bulk density to its poured (loose) bulk density

$$\text{FORMULA: HAUSNER'S RATIO} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of repose

Angle of repose powder poured from a vessel forms a cone-like pile. The angle of repose- the angle between the slope of the pile and the horizontal correlates with the strength of particle- particle interactions and, therefore, is measured to infer flow ability.

$$\text{FORMULA: } \phi = \tan^{-1}(h/r)$$

Where,

- h : the height in cm
- r : the radius in cm
- ϕ : the angle of repose

% Ash value

The ash values usually represent the inorganic residues such as phosphates, carbonates and silicates present in herbal drugs

$$\text{FORMULA: \% ASH} = \frac{w_2 - w_0}{w_1} \times 100$$

- W2: weight of crucible + ash
- W0: weight of crucible
- W1: weight of sample

PHYTOCHEMICAL SCREENING

Qualitative analysis of *Tinospora Cordifolia*

By using the procedure which is given into the standard chart, the following identification *Tinospora Cordifolia* test was performed obtained results were given into the table 1.1

Table 1.1 Preliminary identification tests for phytochemicals in *Tinospora Cordifolia*

Sr.no	Constituent	Chloroform	Methanolic	Ethanolics
		Leaves	Leaves	Leaves
1	Alkaloids	-	+	-
2	Glycosides	-	+	+
3	Flavonoids	+	+	+
4	Phenolics	-	-	-
5	Amino acids	-	-	+
6	Carbohydrates	+	-	-
7	Proteins	-	-	+
8	Saponins	-	-	+
9	Diterpenoids	+	-	+

(Table 1.1)

(+ = Present), (- = Absent)

Above charts shows that the flavonoids, phenolic compounds, tannins, carbohydrates, proteins, alkaloids, fat and oils are present into the Gulvel sample.

Qualitative analysis of *Ocimum sanctum*

By using the procedure which is given into the standard chart, the following identification *Ocimum sanctum* test was performed obtained results were given into the table 1.2.

Table 1.2 Preliminary identification tests for phytochemicals in *Ocimum sanctum*

Sr.no	Constituent	Aq. Extract	Methanolic	Ethanolics
		Leaves	Leaves	Leaves
1	Alkaloids	+	+	+
2	Glycosides	+	+	+
3	Flavonoids	+	+	+
4	Phenolics	+	+	-
5	Amino acids	-	-	-
6	Carbohydrates	-	+	+
7	Proteins	+	+	-
8	Saponins	-	+	+
9	Diterpenoids	-	+	+

(Table 1.2)

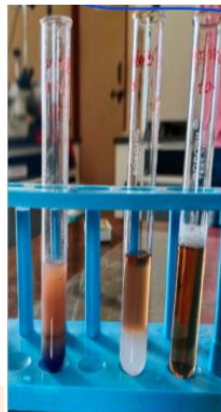
(+ = Present), (- = Absent)

Above charts shows that the flavonoids, phenolic compounds, tannins, carbohydrates, proteins, alkaloids, fat and oils are present into the Tulsi sample.

- Phytochemical Screening of given sample (Test And Result)**

Test	Result
------	--------

Alkaloids Dragendroff's Meyers Wagner's Hager's Murexoid test	Orange red /brown ppt White ppt Reddish brown ppt Yellow ppt Purple colour observed
Glycosides Legal's test Baljet test Borntrager test	Pink red colour Yellow or orange colour Layer showed pink, red or violet colour
Flavonoids Shinoda test Alkaline reagent test	Reddish pink or brown colour observed Yellow colour changed into colourless
Tannin test Test 1 Test 2 Test 3 Test 4	Dark blue greenish black colour appear Deep red colour produce Yellow precipitate formed White precipitate formed
Carbohydrate test Molisch test Benedict's test Fehling's test	Reddish violet and purple ring at junction between two liquid. Solution appear green, yellow and red depending on amount of reducing sugar. First yellow then brick red precipitate is seen.
Monosaccharide test Barfoed's test	Red precipitate is observed

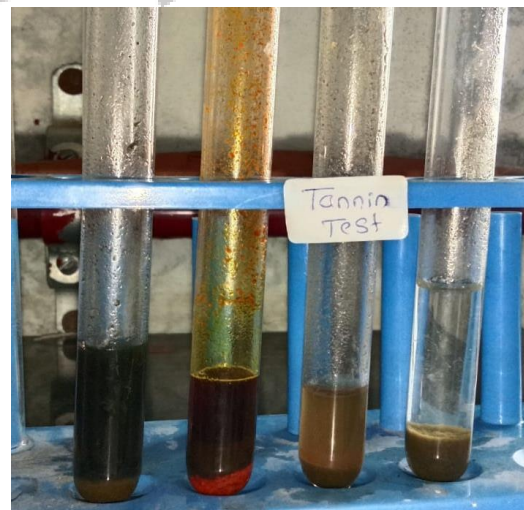
1) Qualitative analysis for *Tinospora Cordifolia***PRELIMINARY TEST FOR
IDENTIFICATION OF PHYTOCHEMICALS****Alkaloid test****Cardiac glycoside test**

(Tannin

Test)

(Alkaloids

Test)



2) Qualitative analysis for *Ocimum sanctum* *Cordifolia*

Quantitative Analysis for *Tinospora*

1) Total Phenolic content (TP) determination

Total phenolic content (TP) was determined by using Gallic acid equivalent method and also another some chemicals was used like Folin-Ciocalteu reagent, 20% sodium carbonate and the results were given into the table 1.1.

Observation table for determination of TPC :-

Sr. No.	Concentration (ug/ml)	Absorbance
1	20	0.932
2	40	1.538
3	60	2.514
4	80	3.162
5	100	3.854

(Table 1.1)

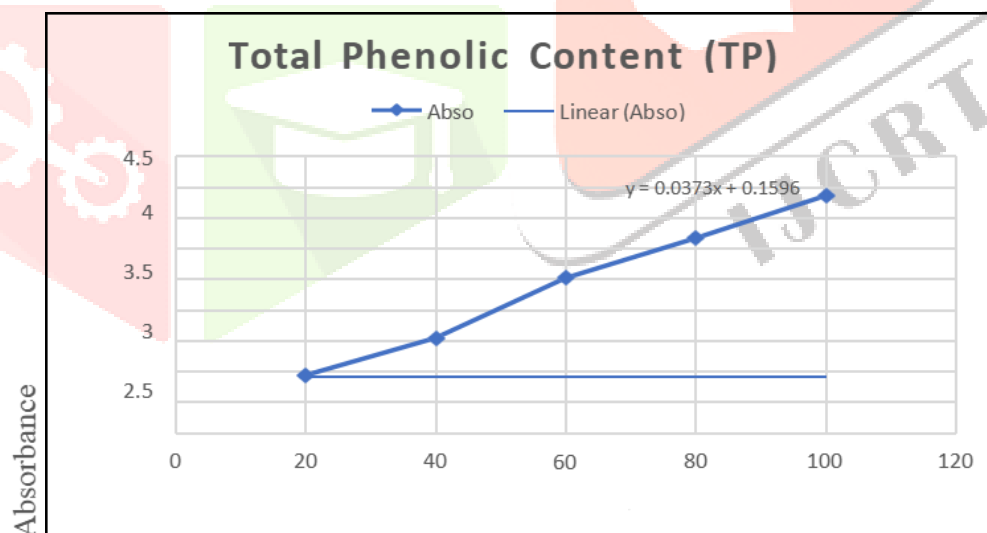


Fig. 1.1. Total phenolic content graph

Discussion - $TPC = C_1 \cdot V/N$ by using this formula we were founded the total phenolic content in the *Tinospora Cordifolia* of was 31.24mg/g.

2) Total Flavonoid content (TF) determination

Total flavonoid content (TF) was determined by using Gallic acid equivalent method and also another some chemicals was used like 5% of NaNO_2 solution, 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution, 1M NaOH and results were given into the table 1.2.

Observation table for determination of TFC :-

Sr. No.	Concentration (ug/ml)	Absorbance
1	0.025	0.512
2	12.5	0.856
3	25	1.1569
4	50	1.8632
5	80	2.3654
6	100	2.6892

(Table 1.2)

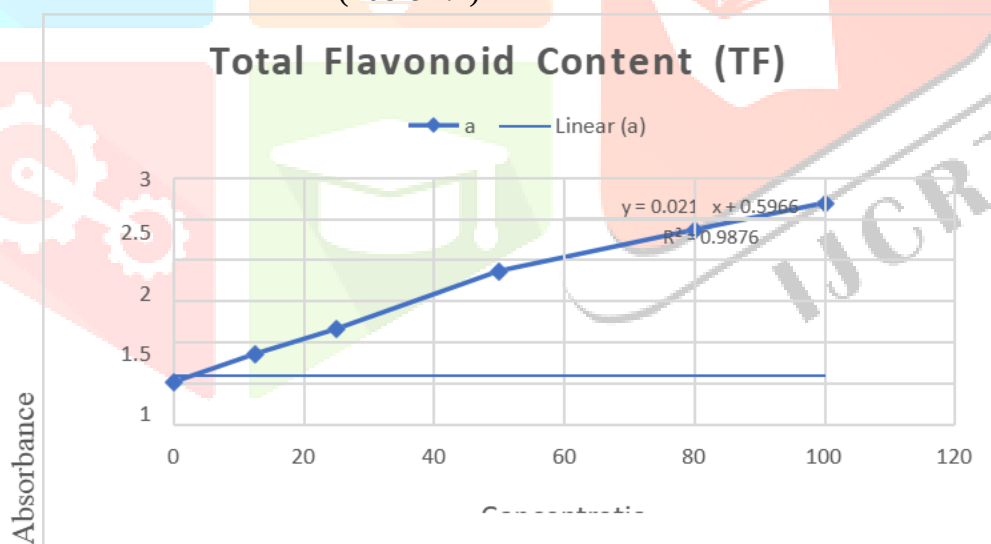


Fig 1.2. Total Flavonoid content graph

Discussion- $\text{TFC} = C_1 \cdot V/N$ by using this formula we were founded the total phenolic content in the leaves extract of *Tinospora Cordifolia* was 22.28mg/g.

3) Total Tannin content (TT) determination

Total tannin content (TT) was determined by using gallic acid equivalent method and also another some chemicals was used like 5% of NaNO_2 solution, 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution, 1M NaOH and results were given into the table 1.3.

Observation table for determination of TTC :-

Sr. No.	Concentration (ug/ml)	Absorbance
1	0.025	0.524
2	12.5	0.967
3	25	1.352
4	50	2.246
5	80	2.598
6	100	2.987

(Table 1.3)

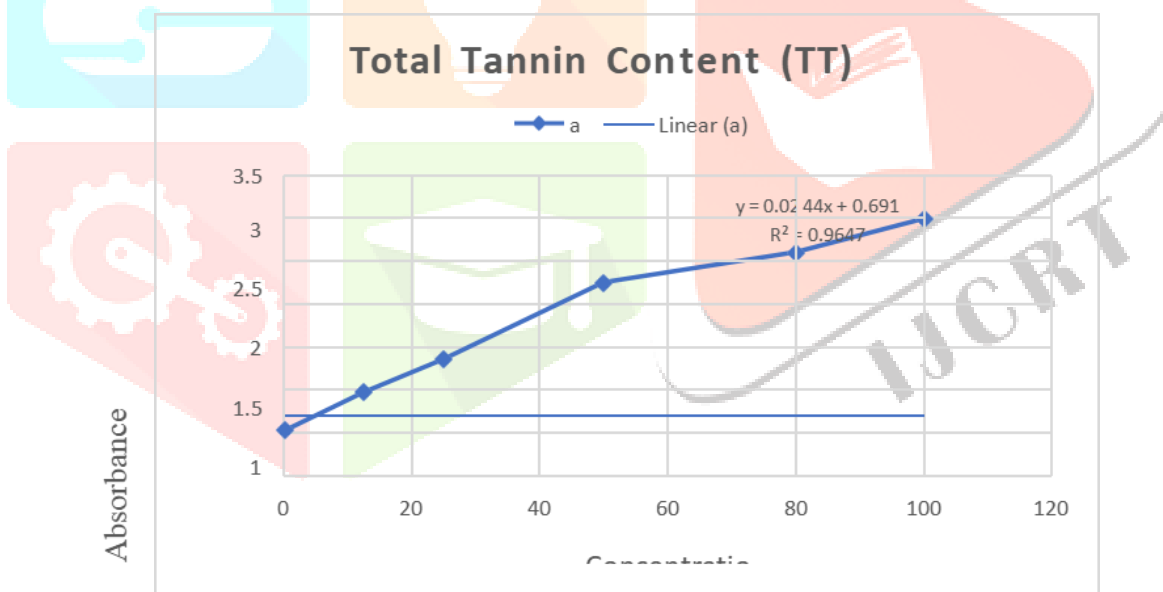


Fig. 1.3. Total Tannin content graph

Discussion- $\text{TFC} = \frac{C_1 \cdot V}{N}$ by using this formula we were founded the total tannin content in the leaves extract of *Tinospora Cordifolia* was 31.24mg/g.

Quantitative Analysis for *Ocimum sanctum*

1) Total Phenolic content (TP) determination

Total phenolic content (TP) was determined by using Gallic acid equivalent method and also another some chemicals was used like Folin-Ciocalteu reagent, 20% sodium carbonate and the results were given into the table 1.4.

Observation table for determination of TPC :-

Sr. No.	Concentration (ug/ml)	Absorbance
1	20	0.930
2	40	1.532
3	60	2.508
4	80	3.160
5	100	3.844

(Table 1.4.)

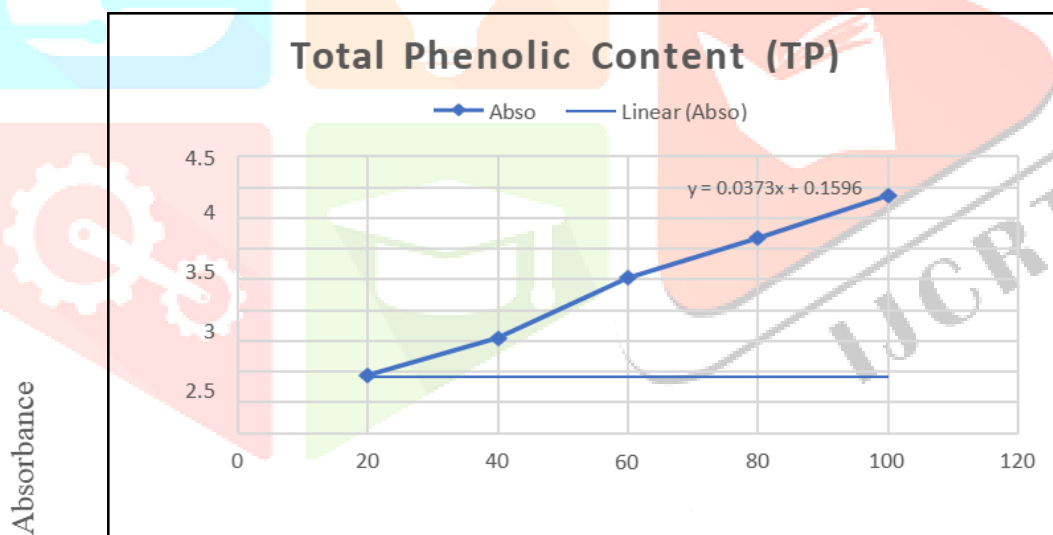


Fig 1.4. Total phenolic content graph

Discussion - $TPC = C1 \cdot V/N$ by using this formula we were founded the total phenolic content in the *Ocimum sanctum* of was 31.15 mg/g.

2) Total Flavonoid content (TF) determination

Total flavonoid content (TF) was determined by using Gallic acid equivalent method and also another some chemicals was used like 5% of NaNO_2 solution, 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution, 1M NaOH and results were given into the table 1.5.

Observation table for determination of TFC :-

Sr. No.	Concentration (ug/ml)	Absorbance
1	0.025	0.510
2	12.5	0.853
3	25	1.1560
4	50	1.8628
5	80	2.3652
6	100	2.6888

(Table 1.5.)

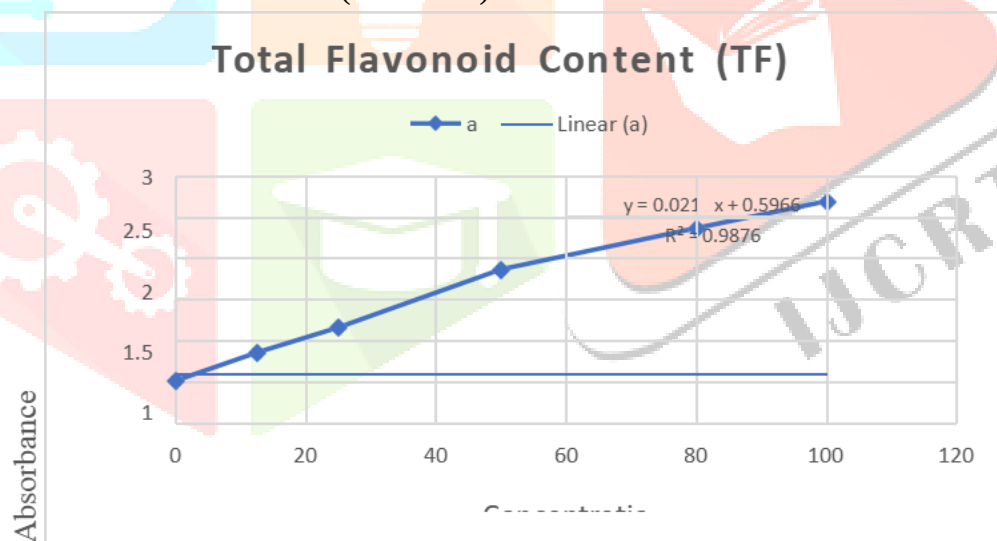


Fig 1.5. Total Flavonoid content graph

Discussion- $\text{TFC} = \frac{C_1 \cdot V}{N}$ by using this formula we were founded the total phenolic content in the leaves extract of *Ocimum sanctum* was 22.24 mg/g.

3) Total Tannin content (TT) determination

Total tannin content (TT) was determined by using gallic acid equivalent method and also another some chemicals was used like 5% of NaNO_2 solution, 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution, 1M NaOH and results were given into the table 1.6.

Observation table for determination of TTC :-

Sr. No.	Concentration (ug/ml)	Absorbance
1	0.025	0.522
2	12.5	0.960
3	25	1.348
4	50	2.240
5	80	2.595
6	100	2.983

(Table 1.6)

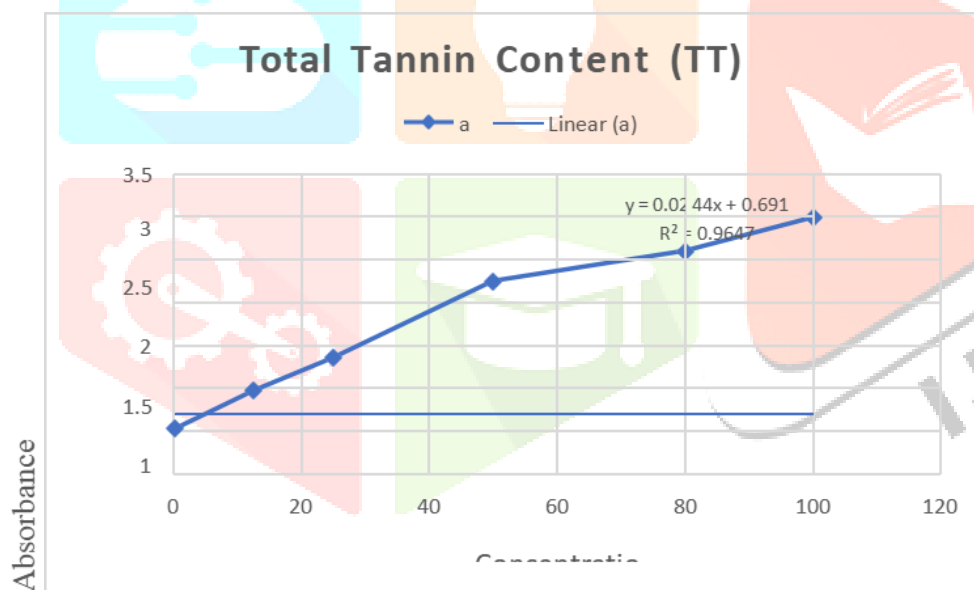


Fig. 1.6. Total Tannin content graph

Discussion- $\text{TFC} = \frac{C_1 \cdot V}{N}$ by using this formula we were founded the total tannin content in the leaves extract of *Ocimum sanctum* was 31.20 mg/g.

ANTIMICROBIAL TESTING

An antimicrobial susceptibility test was carried out against *Escherichia coli* and *Staphylococcus aureus* by Agar cup method. It typically involves evaluating the ability of a substance or product to kill or inhibit the growth of microorganism that may cause infection in a wound.

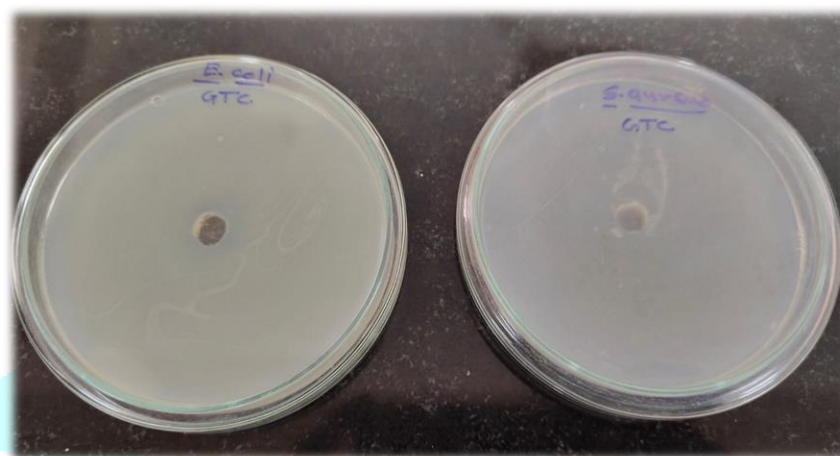
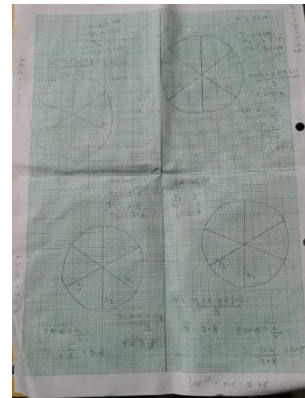


Fig. Zone of inhibition

PREFORMULATION

Parameters	Result
1. Bulk density	0.58g/ml
2. Tapped density	0.25g/ml
3. Carr's index	53.6%
4. Hausner's ratio	2.15
5. Angle of repose	27°92 °c
6. %Ash Value	12%

Table no. 1: Pre formulation of *Tinospora cordifolia* sample



(Bulk Density)

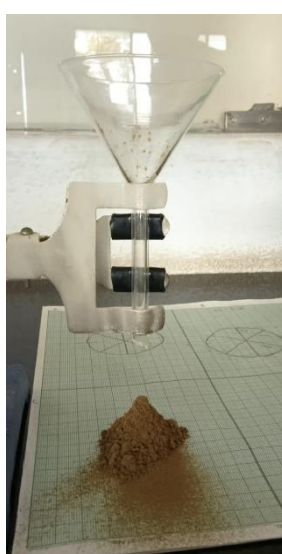
(Tap Density)

(Angle of repose)

(%Ash Value)

Parameters	Result
1. Bulk density	0.28g/ml
2. Tapped density	0.47g/ml
3. Carr's index	59.10%
4. Hausner's ratio	1.67
5. Angle of repose	0.36 °c
6. %Ash Value	15%

Table no. 2: Pre formulation of *Ocimum sanctum* sample



(B.D)

(T.D)

(Angle of repose)

(%Ash value)

FORMULATION OF A WOUND HEALING CREAM

Sr.no.	Composition	Quantity
1	Gulvel extract	4ml
2	Tulsi extract	4ml
3	Beeswax	4gm
4	Liquid paraffin	15ml
5	Borax	0.4gm
6	Methyl paraben	0.04gm
7	Distilled water	7ml
8	Orange oil	1 Drop
	Net.Wt.	34gm

FORMULATION OF CREAM

Procedure

After successful Extraction, the formulation of cream was performed as follows:

Take 10 ml of liquid paraffin and 3 g of beeswax in borosilicated glass beaker. This beaker is heated until the beeswax is completely melted and mixed with liquid paraffin. This is the Oil phase. Take another

beaker in which 0.2 g of borax, 0.02 g of methyl paraben were added to distilled water and heated on a water bath until get clear solution. This is an aqueous phase. Then, slowly add this aqueous phase to the heated oily phase with vigorous shaking. Then add 4 ml of Gulvel extract, 4 ml of tulsi extract to the above mixture and add a few drops of orange oil. Stir this mixture vigorously, then take the ointment slab and pour the mixture on it. After the pouring, take a knife and mix until a smooth cream is formed.



EVALUATION OF DEVELOPED FORMULATION

1. **Washability:** The cream was applied to the hand and then washed with tap water.
2. **Irritancy:** The Formulation was applied on the dorsal surface and waited for 24 hours.
3. **Phase Separation:** The cream was kept in a closed container at room temperature for 24 hours.
4. **Greasiness:** The cream was applied to the dorsal hand, and it was checked for greasiness.
5. **pH:** A small quantity of cream was applied to the pH Paper.
6. **Physical Evaluation:** Physical evaluation involves color, odor, and texture identification.
7. **Antimicrobial Activity:** Prepare agar media. The Formulation was heated lightly and added into the pores of agar and kept for 48 hours in an Incubator. After 48 hours, check the zone of inhibition, which gives information about antimicrobial activity.

RESULTS OF EVALUATION OF DEVELOPED FORMULATION

- 1) **Washability:** The above formulation is washable under tap water.
- 2) **Irritancy:** The above formulation does not show any signs of irritancy.

- 3) **Phase separation:** The above formulation does not show any signs of phase separation.
- 4) **Greasiness:** The above formulation is greasy.
- 5) **pH:** The pH of the above formulation was found to be 7.3.
- 6) **Physical Evaluation:** The above formulation has colour, it does not have odour, and also has a smooth texture.
- 7) **Antimicrobial Activity:** The above formulation shows the zone of inhibition, which indicates the presence of antimicrobial activity.



Fig. Washability



Fig. Irritancy



Fig. pH test

STABILITY TEST

The formulated cream was kept under two different temperature (at room temperature and 4°C) for thirty days following the formulation's first day. Characteristics including the cream's colour, consistency, and homogeneity were noted. The cream sample were stable at both temperature after 25 days as no alteration in the observation were noticed.

NO. OF DAYS	PARAMETERS	4°C	ROOM TEMPERATURE
DAY 1	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 5	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 10	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 15	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 20	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 25	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous

CONCLUSION

The present study successfully carried out the phytochemical analysis and evaluation of a polyherbal cream formulated for wound healing activity. Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which are known to contribute significantly to antimicrobial, anti-inflammatory, and antioxidant activities.

The formulated polyherbal cream showed satisfactory physicochemical properties, including appropriate pH, spreadability, consistency, and stability, indicating its suitability for topical application. Evaluation studies demonstrated that the cream possesses promising wound healing potential, as evidenced by enhanced wound contraction, reduced healing time, and possible antimicrobial effects against common pathogens.

Further studies, including detailed pharmacological and clinical evaluations, are recommended to validate its efficacy and safety for human use.

REFERENCE:-

1. Gundu A, Krishna Sailaja A. An overall review on *Tinospora Cordifolia*. Experimental Medicine. 2022;2(1):1-6.
2. Tiwari P, Nayak P, Prusty SK, Sahu PK, Phytochemistry and pharmacology of *Tinospora Cordifolia*: A review. Systemic Reviews in pharmacy. 2018;9(1):70-8.
3. Pharmacology of *Tinospora Cordifolia*, International Journal of Systemic Reviews in Pharmacy, 2018;9(1):70-78.
4. Reddy NM, Reddy RN, Ready NA. *Tinospora Cordifolia*: Chemical constituents and Medicinal properties: a review. Scholar Academic Journal of Pharmacy. 2015;4(8):364-369.
5. Mittal J, Sharma MM, Batra A. *Tinospora Cordifolia*: a multipurpose medicinal plant: A review. Journal of Medicinal Plants Studies. 2014;2(2):32-47.
6. Amit K. Upadhyay, Kumar K, Arvind Kumar, Harish Mishra, *Tinospora Cordifolia* (Willd) Hook, Thoms. (Guduchi)-Validation of the Ayurvedic Pharmacology Through Experimental and Clinical Studies, International Journal of Ayurvedic Research, 2010;2(1):112-121.
7. Warriar R.R, Singh B.G, Anandalakshmi R, Sivakumar V, Kumar A.M, Shivalingam R. "Vegetative Propagation of *Tinospora cordifolia* - A Folkloric and Ayurvedic Medicinal Plant", Journal of Biomedical, 2007; vol.2(2), pp: 131-137.
8. Jalalpura S.S, Alagawadia K.R, Mahajanshetty C.S. "In Vitro Antihelmintic Property of Various Seed Oils," Iranian Journal of Pharmaceutical Research, 2006; vol.4, pp: 281-284.
9. S S Singh, S C Pandey, S Srivastava, V S Gupta, B Patro & A C Ghosh., Indian J. Pharmacol., 35, 83, 2003.
10. Kumar A, Jnanasha AC, Nagaraju S, Cultivation and medicinal properties of Giloy, *Tinospora Cordifolia* (Thunb.) Miers, 18(1). Pharmacology, 2001: 1(3):5-53.
11. The Wealth of India, Raw Materials; Publication & Information Directorate, Council of Scientific & Industrial Research: New Delhi, 10, 252, 1982.
12. Biswas NP, Biswas AK. Evaluation of some leaf dusts as grain protectant against rice weevil *Sitophilus oryzae* (Linn.) Environ Ecol. 2005; 23:485-8.
13. Chatterjee, Gautam (2001). Sacred Hindu Symbols. Abhinav Publications. pp. 93. ISBN 9788170173977. Simoons, pp. 17-18.

14. Claus, Peter J.; Sarah Diamond, Margaret Ann Mills (2003). South Asian Folklore: An Encyclopedia. Taylor and Francis. p. 619. ISBN 9780415939195.
15. Cor JS, Beach JF, Blair A, Clark AJ, King J, Lee TB, et al. Disodium chromoglycate. Adv Drug Res. 1970; 5:190–6.
16. Devi, P. Uma; Ganasoundari, A.. Modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury. Indian Journal of Experimental Biology, v.37, n.3, 1999. March,:262- 268.
17. Flood, Gavin D. (2001). The Blackwell companion to Hinduism. Wiley-Blackwell. pp. 331. ISBN 9780631215356.
18. Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm Boil. 2001;39:8–17.
19. Jyoti Sethi, Sushma Sood, Shashi Seth, and Anjana Talwar., Evaluation of Hypoglycemic and Antioxidant Effect of *Ocimum Sanctum*., Indian Journal of Clinical Biochemistry, 2004, 19 (2) 152-155.
20. Kothari, S K; Bhattacharya, A K, et al. (November/December 2005). "Volatile Constituents in Oil from Different Plant Parts of Methyl Eugenol-Rich *Ocimum tenuiflorum* L.f. (syn. *O. sanctum* L.) Grown in South India". Journal of Essential Oil Research: JEOR. Retrieved 2008-09-05
21. Kuhn, Merrily; David Winston (2007). Winston and Kuhn's Herbal Therapy and Supplements: A Scientific and Traditional Approach. Lippincott Williams and Wilkins. p. 260. ISBN 9781582554624
22. NIIR Board, National Institute of Industrial Research (India) (2004). Compendium of Medicinal Plants. 2004. National Institute of Industrial Research. p. 320. ISBN 9788186623800.