WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 11, 1401-1429. Research Article ISSN 2277–7105

FORMULATION AND EVALUATION OF HERBAL ANTIFUNGAL GEL OF HEMIDESMUS INDICUS *AND TRIDAX PROCUMBENS*

Sakshi Dipak Patade*, Sakshi M. Patil, Swarangi Ravindra Desai, Sakshi Sardar Shevale and Jeevak B. Naik

Genesis Institute of Pharmacy, Radhanagari, 529/30, Near Gaibbi Titta, Radhanagari, Opposite to Bharat Petrol Pump, Tal- Radhanagari, Dist- Kolhapur, 416212.

Article Received on
09 April 2025,

Revised on 29 April 2025,
Accepted on 19 May 2025

DOI: 10.20959/wjpr202511-36889

***Corresponding Author**
Sakshi Dipak Patade
Genesis Institute of Pharmacy, Radhanagari, 529/30, Near Gaibbi Titta, Radhanagari, Opposite to Bharat Petrol Pump, Tal- Radhanagari, Dist- Kolhapur, 416212.

ABSTRACT

The two notable plants selection was done by studying their various activity. These plants were collected and shade dried for about 15 days. The authentication of two plants was done. Herbal plants extracted by using maceration and soxhalet apparatus respectively. Phytochemical screening was performed by ultraviolet and infrared spectroscopy, by these methods we confimed the presence of functional groups mainly rutin and quercetin which shows mainly antifungal activity. Through studying various antifungal topical dosage form we found that using these herbs *(H.indicus and T. procumbens)* no formulation was prepared. We choosed to prepared gel formulation because theirs fast absorbing property, lightweight and non greasy texture. The gel was formulated using respective herbal extract. After evalution of the gel and MIC of formulation against fungus Candida albicans we found that these two drugs show maximum activity at low concentration i.e they show potent activity (2.5% for 100µg). These drug are easily available. They are used for various diseases treatment (antidiabetis, anticarcinogenic, antiulcer, antiarithritis). These are less toxic and affordable.

KEYWORDS: *Hemidesmus indicus*, *Tridax procumbens*, Antifungal activity, Candida albicans, Herbal Antifungal gel.

INTRODUCTION[1]

Herbal gel is a solid, jelly-like substance that can have properties ranging from soft and weak to hard and tough preparation. It is used topically for a variety of purposes, such as

protectants, antiseptics, and antimicrobials.

Advantage of gel

- The topical gel must not be sticky.
- Non-greasy application.
- Being washable and non-toxic.
- Being easy to formulate with active ingredients.
- Stability over time.
- Easy spreading.
- Ability to target affected area for rapid treatment and relief.
- Preventing unwanted side effects through bypassing the digestive system.

Disadvantages of gel

- Some drugs aren't absorbed easily thoroughly skin.
- There's possibility of an allergic reaction.
- The effect of gel initiates slower.
- The gel may irritate the skin.
- Application site must be monitored for reaction.

Ideal properties

- The gel should be clear, non-cloudy and free from lump.
- It should not react with other things or irritate your skin if you use it on yourself.
- It should spread smoothly on whatever you need to put it on, without being too sticky or messy.
- It should be thick enough to stay where you put it but thin enough to spread easily.
- It should be safe and non-irritating for its intended use.
- It should not get separate into liquid, solid or spoiled easily.

***Hemidesmus indicus*[2]**

Hemidesmus indicus often referred to as Anantmool or Indian Sarsaparilla, is a long-lasting, slim, milky-producing, and twining climbing vine shrub. *Hemidesmus indicus* is a valuable medicinal plant known for its distinctive morphological features, including its twining growth habit, aromatic roots, lanceolate leaves, greenish-purple flowers, and elongated cylindrical fruits. Due to its numerous health benefits, it has been widely used in traditional Ayurveda

and herbal medicine.

Botanical name:-Hemidesmus indicus (L.) R. Br.

Common names:-Anantmool, Indian Sarsaparilla

Biological source:-The woody rootstock of plant *Hemidesmus indicus*

Family:-Apocynaceae

Plant type:-Slender, laticiferous, twining shrub

Geographical source

H. indicus is a creeping or semi-upright shrub that can be found all over India, extending from the upper Gangetic plains, eastward to Assam, and throughout central India. It is also acknowledged to flourish in Malaysia, Indonesia, Bangladesh, and Sri Lanka.

Chemical constituents

Sitosterol, α-amyrin, β-amyrin, β-amyrin acetate, hexatriacontane, lupeol, and lupeol octacosanoate are just a few of the bioactive compounds found in the roots of *Hemidesmus indicus.*

Root system

The plant develops long, cylindrical roots that are slightly twisted and aromatic. The roots have a characteristic pleasant fragrance, making them a key component in traditional medicine. The outer bark of the root is brown, giving it a rough texture.

Fig. 1: Root of *Hemidesmus Indicus.*

Stem and Growth Pattern

The stem of *Hemidesmus indicus* is slender, with nodes that are slightly thickened. The plant exhibits a twining and climbing habit, often using other plants or structures for support.

Leaf morphology

The leaves of Anantmool are simple, opposite, and they lack stipules. They are shortly petioled and exhibit an entire margin with a smooth surface. Each leaf has a prominent white-colored striation down the middle. The mature leaves are generally broad lanceolate in shape. The leaves typically range from 5 to 10 cm in length and are dark green with a distinct reticulate venation pattern.

Flowers and Inflorescence

The blooms of *Hemidesmus indicus* are tiny and fragile, displaying a greenish-purple hue. They are arranged in sub-sessile cymes, which are compact clusters of flowers found in the axils of the leaves in an opposite pattern.

Fruits and Seeds

The fruits are elongated, cylindrical pods that may reach lengths of up to 10 cm. The seeds are oblong and flat, with a tuft of white silky hair at one end. This specialized structure aids in seed dispersal through wind, allowing the plant to propagate efficiently in its natural habitat.

Taxonomical classification of *hemidesmus indicus*[2]

Table no. 1: Taxonomical classification of *Hemidesmus indicus.*

Kingdom	**Plantae**
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Gentianales
Family	Asclepiadaceae
Genus	Hemidesmus
Species	Indicus

Uses of *hemidesmus indicus*

- It is believed to be beneficial for skin conditions, excessive menstrual bleeding, and recovery after childbirth, stomach ulcers, and other digestive problems.
- This plant has been valued in folk medicine for treating venereal diseases like gonorrhea and syphilis, as well as used to relieve fever, headaches, and mouth sores.
- They are used to manage poor appetite, fever, skin disorders, white discharge in women (leucorrhoea), syphilis, and joint pain from rheumatism.
- Scientific studies have shown that the root has strong antimicrobial and anti-inflammatory

properties.

***Tridax procumbens*[3]**

Tridax procumbens also known as coat buttons or tridax daisy, is a flowering plant from the sunflower family (Asteraceae). It is recognized as an invasive weed and a problematic plant This plant has daisy-like flowers with a yellow center and white or yellow petals that have three- toothed tips. The leaves are arrowhead-shaped with serrated edges. The calyx is very small or changed into a pappus (hair-like structure that helps in seed dispersal).

Botanical name:- Tridax Procumbens, Chrysanthemum procumbens (L.)

Common name:- Jayanti Veda, Ghamra

Biological source:-The entire plant, a widespread herbaceous weed.

Family:-Asteraceae

Plant type:-semi-prostrate or procumbent herb.

Geographical source

T. procumbens is a wild herb found across India, Nepal, and Nigeria that is utilized for the treatment of bronchial catarrh, diarrhea, and inflammation. It is an annual or perennial plant, native to Central and South America and occurs throughout India as a weed, commonly known as 'Coat buttons' and tridax daisy in English.

Chemical constituent

Tridax procumbens contains alkaloids, flavonoids, carotenoids, saponins, tannins, essential oils, and terpenoids, with some studies highlighting specific compounds like procumbent, beta- sitosterol, and various fatty acids. The plant also contains compounds like benzoic acid derivatives (e.g., ferulic acid), lignans (e.g., galgravin), and polysaccharides.

Specific compounds

Procumbenetin: A flavonoid isolated from the aerial parts of the plant. Beta-sitosterol: A phytosterol. Oleanolic acid, puerarin, esculetin, and betulinic acid: These compounds have been isolated from the plant parts.

Fig. 2:- Leaves of *Tridax Procumbens.*

Plant morphology

Tridax procumbens:-Morphological Characteristics

Growth habit

Tridax procumbens has a trailing or creeping growth pattern. Instead of growing upright, it extends horizontally across the ground. This sprawling nature allows it to cover the soil surface effectively, making it a common ground cover in various regions. Its ability to spread laterally helps to establish itself in different environments, particularly in disturbed areas such as roadsides, lawns, and agricultural fields.

Stem characteristics

The stalk of *Tridax Procumbens* is tubular in form and adorned with harsh, multicellular fibers, imparting a coarse feel. The stem functions as strong transport system for water, nutrients, and food. It exhibits an ascending growth habit, reaching a height of approximately 30 to 50 centuries, and is sparsely branched. The stem are somewhat flexible and thin, adapting to their creeping or trailing nature, which enable the plant to spread across the ground and form a mat-like structure.

Root system

As a low-growing species, *Tridax procumbens* possesses a shallow root system, primarily concentrated near the soil surface. This root structure allows for efficient absorption of moisture and nutrients from the top layer of the soil and also prevent easy uprooted by wind and soil. The ability to root at nodes further enhances its stability and spread, making it a persistent species in its habitat.

Flower structure

The flowers of *Tridax procumbens* resemble those of a daisy, featuring a distinctive composite structure. Each blossom is made up of two kinds of florets: ray florets and disc florets. The ray florets, which form the outermost part of the flower head, have three-lobed or toothed petal-like structures that can be either white or yellow. The disc florets, located at the center, are small, tubular, and yellow, giving the flower its characteristic daisy-like appearance. The long flowering period also makes it a valuable nectar source for pollinators, including bees and butterflies.

Leaf morphology

The leaves of *Tridax procumbens* are simple in structure, meaning they are not divided into separate leaflets. They exhibit triangular shape, with irregularly toothed margins**.** The leaves are oppositely arranged on the stem, meaning that pairs of leaves emerge at the same level but on opposite sides of the stem. The leaf size generally ranges between 3 to 7 cm in length.

Taxonomical classification of *tridax procumbens*[3]

Table no. 2: Taxonomical classification of *Tridax procumbens.*

Kingdom	**Plantae**
Subkingdom	Tracheobionata
Division	Spermatophyta
Subdivision	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	Tridax
Species	Procumbens

Uses of *tridax procumbens*

- *Tridax procumbens* has the ability to fight bacteria, protozoa, and fungi, making it useful in treating various infections.
- The juice from its leaves is traditionally used to heal wounds, especially deep or internal wounds. Its seeds are used to stop different types of bleeding
- It is also used to treat muscle cramps and is considered a safe ingredient for future medicinal use.
- *Tridax procumbens* also has other health benefits, including boosting the immune system, controlling blood sugar, protecting the liver, and providing antioxidant, anti-inflammatory, and pain-relieving effects.

Introduction about Fungal Effect[4]

Human skin serves as a protective barrier, yet it can sometimes become infected by fungi. These infections can transfer through common behaviors such as wearing tight cloths or sharking locker rooms, clothing, furniture or with an infected individual. Fungal infection typically present symptoms like red, itchy patches on skin, hair loss, and crusty region. To combat fungal infection physician prescribe antifungal medications available in various forms, including creams, oral tablets, and intravenous treatments. However, oral antifungal medication generally has a higher toxicity than topical creams. Azole function by inhibiting particular enzyme in fungal cell membrane, which hinders the fungus from producing sterol (ergosterol), a necessary component for its survival. Without ergosterol, the fungus cell wall become fragile and permits substances to flow through.

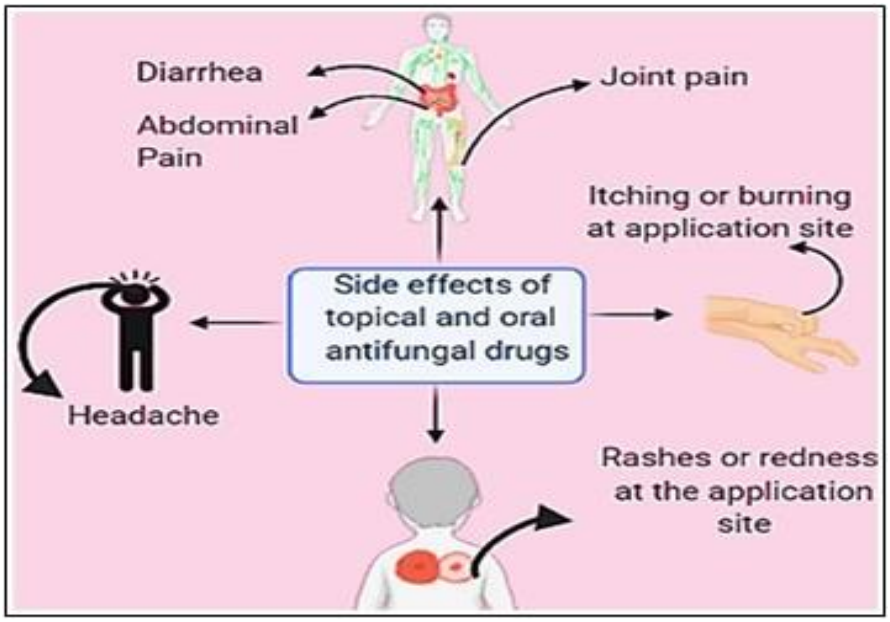


Fig. 3: Side effect of synthetic antifungal drug.

Antifungal activity[4]

Antifungal activity refers to the process of inhibiting fungal pathogens through extracts. Antifungal medications specifically eliminate fungal infections in a host while minimizing harm to the host. These drugs aim at structures or functions vital to fungal cells but absent in human cells, allowing them to combat a fungal infection without harming the cells of your body. The fungal cell membrane and the fungal cell wall are two structures that are often targeted. Antifungals function by attaching to specific elements of the fungal plasma membrane or its biosynthetic pathways or even components of the cell wall.

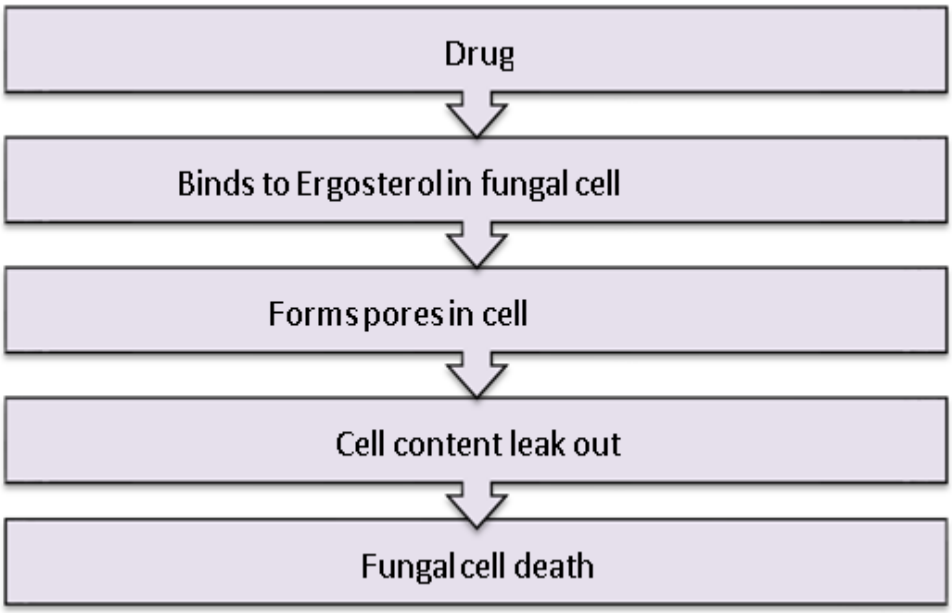


Fig. 4: Mechanism of antifungal drug.

Literature review

1. ***Rituraj Rajjan Singh*** *et.al.(2024)* have explained the dried methanolic extract of herbs was used to make the gels. It is applied topically for a number of reasons, including antimicrobials, antiseptics, and protectants. The herbal gel was found to have the greatest impact on hand bacteria. Further research is necessary to completely comprehend the mechanism of action and to develop a formulation that can be helpful in the health sector.

2. ***Vaibhav Ramdas Jadhav*** *et.al. (2024)* have investigated that *Tridax procumbens* Linn, a tropical American weed, has numerous pharmacological activities including liver protection, immunomodulation, wound healing, and antimicrobial properties. It has also been used as a bioadsorbent for chromium removal. However, scientific research linking its phytochemical constituents with its pharmacological properties is limited.

3. ***Shravani R Kakad*** *et.al. (2024)* state that this review article provides detailed information on *Hemidesmus indicus*, a plant known for its potential against various diseases and its biological and pharmacological activities. It highlights its species, botanical name, taxonomical classification, morphology, chemical constituents, and traditional uses, providing valuable insights for future researchers.

4. ***Kalpana S. Patil*** *et.al (2023)* have developed that Topical medications are commonly used to treat skin conditions, but antibiotic-resistant bacteria have led to a search for natural healing methods. Cocculu shirsutus and *Tridax procumbens* leaves were

authenticated, dried, and extracted, and their extracts were analyzed for antimicrobial and anti- inflammatory properties. A polyherbal gel was formulated using different agents, with the F4 formulation showing the best results.

5. ***Debolina Dattaray*** *et.al (2022)* states that *Tridax procumbens*, an annual herb, is used in Indian traditional medicine due to its medicinal properties and bioactive constituents. The plant's secondary metabolites, including tannins, alkaloids, saponins, flavonoids, and proteins, have been found to have antibacterial, anti-inflammatory, anti-oxidant, anti-diabetic, and hypotensive activities.

6. ***Suraj Mandal*** *et.al. (2021)* have explained that the research focuses on developing a topical gel formulation of fluconazole using natural Aloe Vera gel, a Triazoles derivative used to prevent and treat candidiasis, to improve patient compliance, reduce drug dose, avoid side effects, and be safe during pregnancy.

7. ***Navkiranjeet Kaur*** *et.al. (2021)* have studied that fungal infections on the skin's third layer can be treated with Azole derivative-based creams, herbal extracts, and essential oils. Polysaccharide-based nanohydrogels, embedded with plant extracts and oils, offer effective delivery agents and protection against side effects.

8. ***Hemlata Kaurav*** *et.al. (2021)* have developed that the Ayurvedic Science field is growing globally due to its medicinal uses. *Hemidesmus indicus*, also known as Anantmool or Indian Sarsaparilla, is a significant medicinal plant with numerous remedial properties. It belongs to the Apocynaceae family and has been used for centuries in Ayurvedic medicine. The plant's parts, roots, and rhizome have been used for various diseases, including anti- cataractous, anti-diarrhoeal, anti-cancerous, anti-diabetics, anti-venom, and anti- angiogenic.

9. ***Vignesh Kanna Balaji*** *et.al. (2017)* tells that Plants have been used in traditional medicines for thousands of years, providing new remedies. *Hemidesmus indicus* root is a plant drug used for tonic, demulcent, diaphoretic, diuretic, and urinary disorders. This study aims to screen phytochemicals and quantify *Hemidesmus indicus* root, providing valuable information for future research.

10. ***S. Nagaraju*** *et.al.(2015)* have explained that *Tridax procumbens* is a plant used in Indian traditional medicine for its medicinal properties, including antioxidant, anti-bacterial,

anti- inflammatory, and vasorelaxant properties. Extracts from the plant are used to treat diabetes, arthritis, and inflammatory reactions, but further studies are needed to isolate and evaluate active principles.

11. ***Sowmya B. Jhample*** *et.al. (2015)* have investigated that the Indian traditional medicinal system, based on Ayurveda, is gaining interest worldwide for its potential in natural bioactive components. Plant extracts have been used in traditional medicine for thousands of years, with many being derived from natural sources. This study explores the bioactive phytoconstituents and antibacterial potential of *Tridax procumbens* L. from Kolhapur district, India.

Need of investigation

Worldwide, approximately 6.5 million individuals suffer from invasive fungal infections each year, resulting in around 3.8 million fatalities. Candida infections alone are responsible for nearly 1 million deaths each year and affect 1.57 million people globally, exhibiting a mortality rate of 63.6%. Serious fungal diseases are estimated to affect about 57 million individuals in India, with significant prevalence of conditions such as recurrent vulvovaginal candidiasis and allergic bronchopulmonary aspergillosis.

- Global Incidence: Annually, there are 6.5 million cases of invasive fungal infections.
- Global Mortality: Invasive fungal infections are responsible for 3.8 million deaths worldwide.
- Candida Infections: Approximately 1.57 million people are affected, resulting in 995,000 fatalities.
- India: Serious fungal diseases impact 57 million individuals.
- Prevalence in India: There are 24.3 million cases of recurrent vulvovaginal candidiasis, 2 million cases of allergic bronchopulmonary aspergillosis, and 25 million school-age children with tinea capitis.
- Annual Incidence in India: Pneumocystis pneumonia occurs in 58,400 cases, invasive aspergillosis in 250,900 cases, mucormycosis in 195,000 cases, and esophageal candidiasis in HIV patients in 266,600 cases.

Gel formulations offer several advantages for treating antifungal infections, Including

- Improved drug delivery,
- Reduced systemic side effects

- Increased patient compliance.

They are more stable and help to remove fungal infection readily from the skin as compared to creams and ointments, so by using *Hemidesmus Indicus* & *Tridax procumbens* Herbal gel formulation we can treat the fungal infection which contains medicinal properties including being antifungal as well as anti-inflammatory, antioxidant, and antimicrobial.

Aim

To formulate and evaluate herbal antifungal gel of Hemidesmus indicus and Tridax procumbens.

Objectives

1. To formulate an antifungal gel *of Hemidesmus indicus* and *Tridax procumbens*.
2. To evaluate antifungal activity of prepared gel.
3. To treat fungal infections of skin, nails by utilizing natural herbs.
4. To provide a safer alternative to synthetic topical application.
5. To formulate a stable, non-greasy and easy topical applicable herbal gel.

Materials

Table no. 3:- List of Material used for Research.

Sr. no.	Material	Sources
1.	Hemidesmus indicus	Radhanagari Wild Life sanctuaries
2.	Tridax procumbens	Radhanagari Wild Life sanctuaries
3.	Carbapol 934	Loba Chemie Pvt. Ltd
4.	Methyl paraben	Loba Chemie Pvt. Ltd
5.	EDTA	Loba Chemie Pvt. Ltd
6.	Propylene glycol	Loba Chemie Pvt. Ltd
7.	Glycerol	Loba Chemie Pvt. Ltd
8.	Triethanolamine	Loba Chemie Pvt. Ltd
9.	Sodium benzoate	Loba Chemie Pvt. Ltd
10.	Distilled water	Collage Laboratory

Table no. 4:- List of instruments/equipment used for Research.

Sr. no.	Instruments/ Equipment	Sources
1.	Magnetic Stirrer	Remi
2.	Weighing Balance	Shimadzu
3.	Water bath	Collage Laboratory
4.	Mortar pestle	Collage Laboratory
5.	pH meter	Eutech

Methods[5]

Collection of plant material

Hemidesmus indicus

The roots of *Hemidesmus indicus* were obtained from the Radhanagari region, Kolhapur, Maharashtra. The gathered plant material was recognized by the department of Pharmacognosy. The roots were rinsed with drinking water 2-3 times and once with sterile distilled water, followed by drying, then a uniform fine powder was produced and kept in an airtight container until needed.

Tridax procumbens

Tridax procumbens leaves were gathered from the Kolhapur area, Maharashtra. The leaves underwent washing with drinkable water 2-3 times and once with sterile distilled water before being dried. The obtained plant material was identified.

Authentification of plants

This authentication certificate verifies the identity of the plant specimen as *Hemidesmus indicus* and *Tridax procumbens*, a roots and leaves species belonging to the family Apocynaceae and Asteraceae respectively. The verification process involved a thorough examination of the plants physical attributes, comparison with reference materials, and taxonomic research to confirm its identity. This certificate serves as official documentation of the plant's authenticity, providing assurance of its identity for scientific, research, or commercial purposes.

The certificate is issued by Doodhasakhar Mahavidyalya Bidri reputable authority in plant taxonomy and identification, and is signed by Dr.S.S.Patil head of botany department a qualified expert in the field. The certificate number and date of issue are also included for reference.

Extraction of plant

Fig. 5: Extraction of *H. Indicus.*

Fig. 6: Extraction of *T. procumbens.*

After the roots of *Hemidesmus indicus* were authenticated, they were shade dried for nearly 1 month. The roots were griend into a coarse powder. For future use, the powder was stored in an airtight glass container. For the preparation of the root extract powdered root (25 g) were soaked in 100 mi each of organic solvent (methanol) and kept on rotating shaker for 72 hours and filtered using Whatman filter paper No.1. The extract were concentrated to half of its volume using electric water bath.

Similarly, the leaves of *Tridax procumbens* were examined, and they were dried in a shaded area for fifteen days. The leaves were pulverized into a rough powder. To prepare for future use, the powder was kept in an airtight glass container. *T. procumbens* dried leaf powder was soaked in methanol for 48 hours prior to being extracted using the Soxhlet apparatus with methanol as the solvent.

Phytochemical screening

Test for carbohydrates

Molisch's test

1 ml of extract was treated with few drops of Molisch's reagent and few drops of concentrated H2SO4 from the side of the test tube; formation of violet ring at the junction of two layers indicates the presence of carbohydrate.

Fehling's test

1ml of filtrate boiled with 1ml each of Fehling's solution A and B Formation of red ppt indicates the presence of sugar.

Barfoed's test

1ml of extract with 1ml of Barfoed's reagent on heating 2min red ppt indicates the presence of sugar.

Benedict's test

1 ml of extract was treated with Benedict's reagent and Boiled for few minutes and observed for the formation of red precipitate indicating the presence of carbohydrates.

Test for alkaloids

Mayer's test

Add a few drops of Mayer's reagent to 1 mL of sample solution. A yellowish or white precipitate indicates the presence of alkaloids. For a more specific test, add 2 mL of concentrated HCl to 2 mL of the sample solution, then add a few drops of Mayer's reagent. A white precipitate or green color indicates the presence of alkaloids.

Dragendorff's test

Add 1 mL of Dragendorff's reagent to 2 mL of sample solution. An orange red precipitate indicates the presence of alkaloids.

Wagner's test

Add a few drops of a solution of 2 g potassium iodide and 1.27 g iodine in 5 mL distilled water, diluted to 100 mL with distilled water, to the sample solution. A brown coloured precipitate indicates the presence of alkaloids.

Hager's test

Treat 2 mL of extract with a few drops of Hager's reagent. A yellow precipitate indicates the presence of alkaloids.

Tests for flavonoids

Sodium hydroxide test

Add a few drops of dilute sodium hydroxide to a 1 mL sample solution in a test tube. An intense yellow color will appear, which will turn colorless when a few drops of dilute acid are added.

Lead acetate test

Add a few drops of 10% lead acetate solution to 10 mg of extract. A yellow colour precipitate

indicates the presence of flavonoids.

Shinoda test

Add 10 drops of dilute hydrochloric acid and a piece of magnesium to 1 ml of sample solution. A deep pink colour indicates the presence of flavonoids.

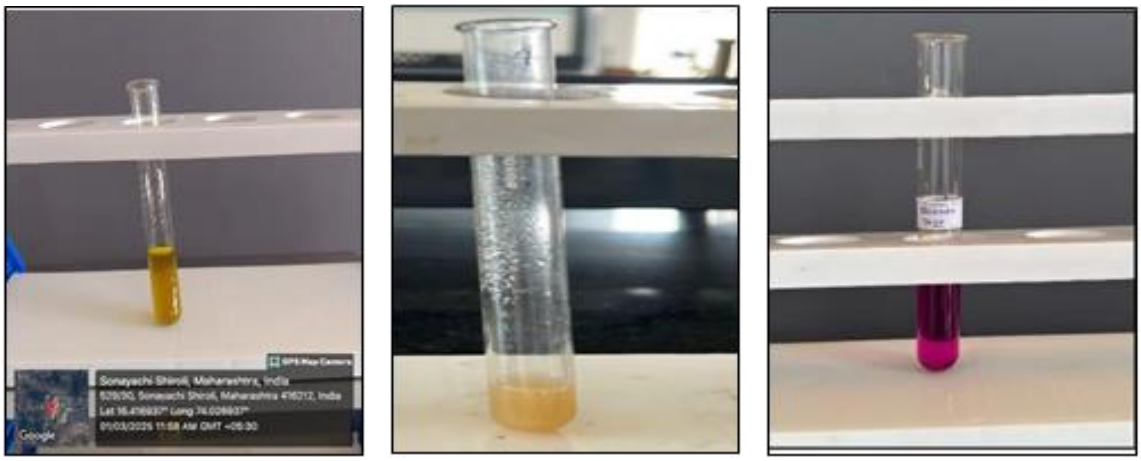


Fig. 7: Test of flavonoids.

Tests for saponins

Ferric chloride test

Two millilitres (2 mL) of the aqueous solution of the extract were added to a few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue colour showed the presence of gallic tannins and a green-blackish colour indicated presence of catechol tannins.

Frothing test

Three millilitres (3 mL) of the aqueous solution of the extract were mixed with 10 mL of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 min, it was allowed to stand for 30 min and observed for honeycomb froth, which was indicative of the presence of saponins.

Foam test

1ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins.

Phytochemical screening of *hemidesmus indicus*

Table no. 5: Phytochemical screening of *Hemidesmus indicus.*

Sr. no	Phytochemical constituents	Type of test	Result
1.	Carbohydrates	Molisch's test	Present
		Fehling's test	Present
		Barfoed's test	Present
		Benedict's test	Present
2.	Alkaloids	Mayer's test	Present
		Dragondroff's test	Present
		Wagner's test	Present
		Hager's test	Present
3.	Flavonoids	Sodium hydroxide test	Present
		Lead acetate test	Present
		Shinod's test	Present
4.	Saponins	Ferric chloride test	Present
		Frothing test	Present
		Foam test	Present

Phytochemical Screening of *Tridax procumbens*

Table no. 6: Phytochemical screening of *Tridax procumbens.*

Sr. no.	Phytochemical Constituents	Results
1.	Alkaloids	Present
2.	Saponins	Present
3.	Flavonoids	Present
4.	Phenols	Present
5.	Carbohydrates	Present
6.	Proteins	Present

Excipient compatibility study

Carbopol 934

Carbopol 934 is cross-linked with allyl sucrose and polymerized in benzene. Carbopol 71, 971, and 974 are crosslinked with allyl pentaerythritol and polymerized in ethyl acetate. Carbomer polymers contain 56%– 68% of carboxylic acid (– COOH) groups and 0.75%– 2% of cross- linking agents.

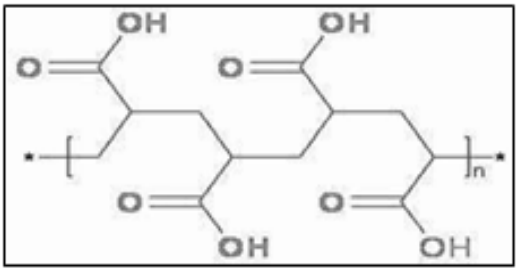


Fig. 8: Structure of Carbopol 934.

Triethanolamine

Triethanolamine (TEA or TEOA) is an oily, viscous organic chemical compound that is a tertiary amine and a triol (a molecule with three alcohol groups). TEA is a bi functional compound that exhibits both properties of alcohols and amines and is used to make surfactants in industrial and cosmetics as a pH adjuster for skin and hair.

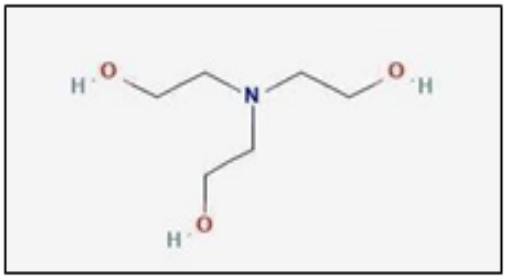


Fig. 9:- Structure of TEA.

Methyl paraben

Methylparaben, also methyl paraben, one of the parabens, is a preservative with the chemical formula CH_3. It is the methyle sterof p-hydroxybenzoic acid.

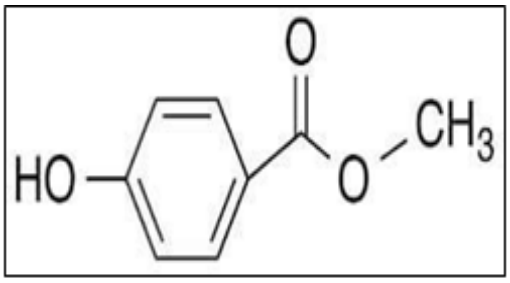


Fig. 10: Structure of methyl paraben.

Propylene glycol

Propylene glycol is a humectant and is widely used in cosmetics and skincare to increase the moisture retention capacity of the products. It is a colorless, viscous liquid that is nearly odorless and has a faintly sweet taste. Formula of Propylene glycol is $C_3H_8O_2$.

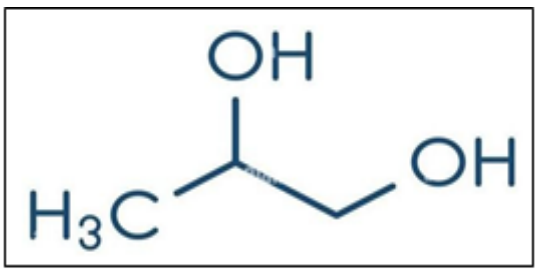


Fig. 11: Structure of Propylene glycol.

Edta

Ethylenediaminetetraacetic acid (EDTA) is a chelating agent can bind to metals via four carboxylate and two amine groups. It is a polyamino carboxylic acid and a colorless, water-soluble solid, which is widely used to dissolve lime scale.

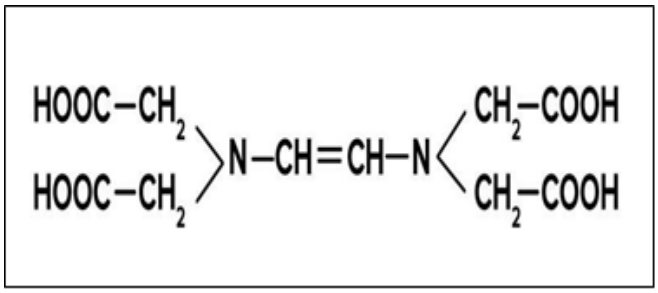


Fig. 12: Structure of EDTA.

Sodium benzoate

Due to its antifungal properties, it helps prevent the growth of bacteria, fungi, and yeast, thus extending the shelf life of products.

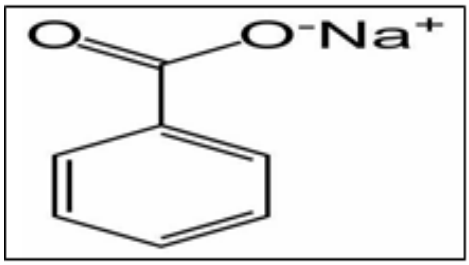


Fig. 13: Structure of sodium benzoate.

Glycerol

Glycerol helps to increase the viscosity of liquid drug formulations, pharmaceutical syrups, etc. It also provides humidity or moisture to drug pills or tablets. Glycerol is used in cement compounds, caulking compounds, and pressure media.

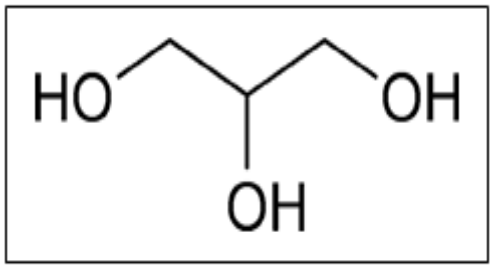


Fig. 14: Structure of glycerol.

Formulation of Antifungal gel containing methanolic extract of *H.indicus* and *Tridax procumbens*

Table no. 7:- Formulae of formulation of Antifungal gel.

Sr no.	Ingredients	Quantity
1.	Hemidesmus indicus	0.3g
2.	Tridax procumbens	0.2g
3.	Carbapol 934	0.3g
4.	Methyl paraben	0.02g
5.	EDTA	0.01g
6.	Propylene glycol	1.6g
7.	Glycerol	1g
8.	Triethanolamine	q.s
9.	Sodium benzoate	0.04g
10.	Distilled water	q.s

Formulation procedure

- ✓ Accurately weighed Carbopol 934 was taken in a beaker and dispersed in 10 ml of distilled water.
- ✓ Kept the beaker aside to swell the Carbopol for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min.
- ✓ Take 1.6g propylene glycol in another beaker and add weighed quantity of methylparaben to it and stirred properly.
- ✓ After all Carbopol dispersed, extract and sodium benzoate, EDTA and glycerol were added with constant stirring.
- ✓ Finally make up the volume upto 20ml by adding remaining distilled water and Triethanolamine was added drop wise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

Fig. 15: Formulation of gel.

Evaluation tests for herbal gel

Physical evaluation of gel

- **Color:-**The color of the formulation was checked out against white Background.
- **Odour:-**The odour of the gel was checked by mixing the gel in water and taking the smell.
- **Consistency:-**The consistency was checked by applying on skin.
- **Greasiness:-**The greasiness was assessed by the application on to the skin
- **Grittiness:-**The formulation was evaluated microscopically under 40x magnifications for the presence of any particulate matter OR aggregates.
- **Homogeneity:-**Homogeneity was tested by visual inspection after allowing them to set in a container. They were evaluated for their appearance and presence of aggregates.

pH Determination

About 20mg of the formulation was taken in a beaker and was subjected to the pH measurement using a digital pH meter within 24hrs. of manufacture.

Spreadability

The spreadability of the formulation was measured by spreading of 0.5 g of the gel on a circle of 2 cm diameter pre marked on a glass plate and then a second glass plate was employed. Half kilogram of weight was permitted to rest on the upper glass plate for 5 min. The diameter of the circle after spreading of the gel was determined.

Formula:-S = M. L / T Where,

M = weight tied to upper slide L = length of glass slides

T = time taken to separate the slides

Viscosity test

The viscosity of the prepared gel was measured by using Brookfield Viscometer. Prepared the gel with different concentration. Setup the base level of the instrument using level indicator. The spindle was cleaned and attached to the instrument. Then the spindle was rotated in the gel until a constant reading displaced on the viscometer.

Stability study

Stability study Physical stability test of the herbal gel was carried out for four weeks at various temperature conditions like 20C, 250C and 370C. The herbal gel was found to be physically stable at different temperature i.e. 20C, 250C, 370C within four weeks.

Antifungal activity of topical agent

Sample Coding 2.5 %, 5%, 10% Antifungal Activity of Topical agent was determined against Candida Albicans by Agar well diffusion assay, in various concentration i.e. 25µg, 50µg, 75µg and 100µg of topical agent directly taken from final formulation aseptically by sterile. Spatula and loaded in wells which are prepared by using sterile cork borer on MGYP plates having diameter 0.7cm. After that plates were keep at 270C for 24 hrs. For incubation, after incubation plates were observed for zone of inhibition around the well.

Characterization of Isolated component obtained from *Hemidesmus Indicus* and Tridax procumbens

UV

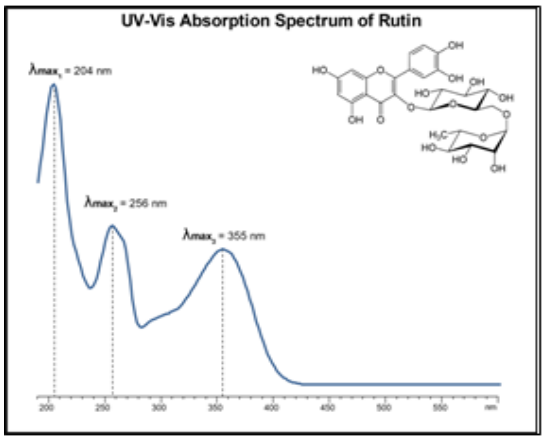


Fig. 16:- UV of Std rutin for comparison.

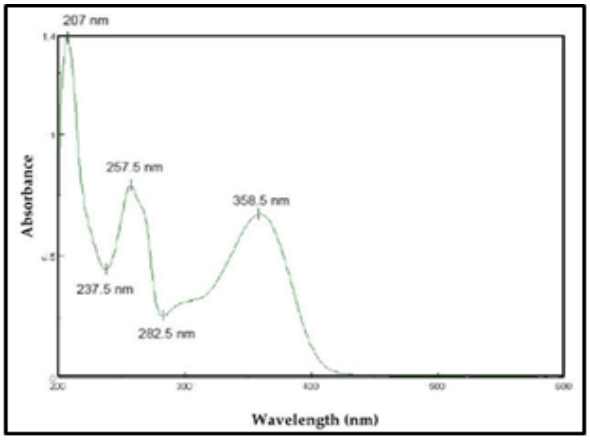


Fig. 17:- UV of Isolated Component of Rutin Obtained from *Hemidesmus Indicus.*

Fig no.16 & 17 Indicates presence of rutin in sample because λmax of std rutin is 355 nmapproximately matching with λmax of sample is 358.5nm of isolated component obtained from *Hemidesmus indicus*.

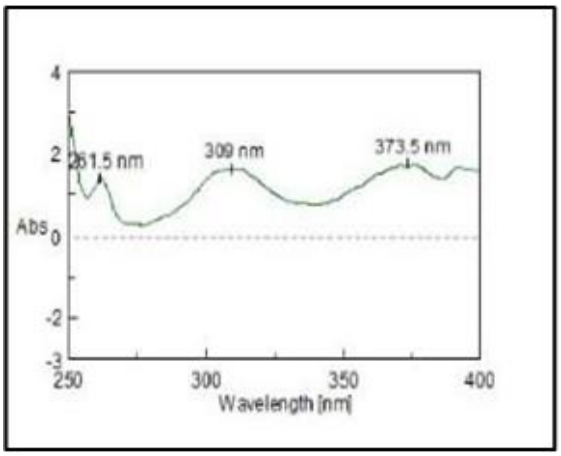


Fig. 18: UV of Std Quercetin for comparison.

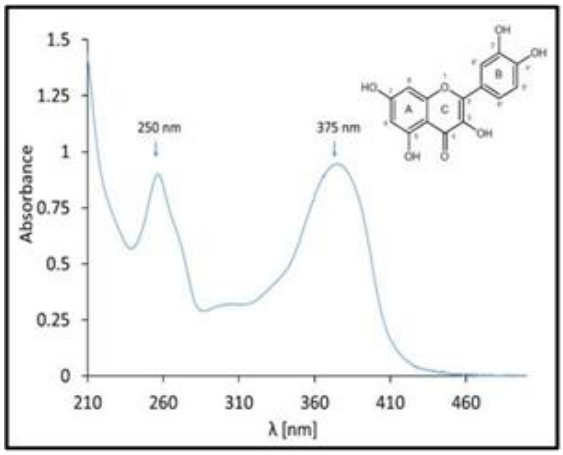


Fig. 19: UV of Isolated Component of Quercetin from *Tridax procumbens.*

Fig no.18 & 19 Indicates presence of rutin in sample because λmax of std rutin is 373.5 nm approximately matching with λmax of sample is 375nm of isolated component obtained from *Tridax procumbens.*

IR

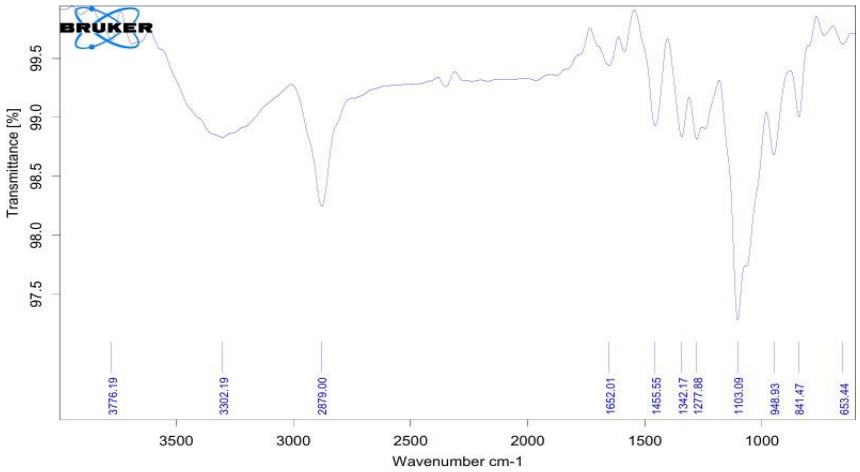


Fig. 20: FTIR of isolated component (Rutin) obtained from *Hemidesmus indicus.*

Table No. 8: FTIR data of isolated component from *Hemidesmus indicus.*

Sr no.	Standard Wavelength	Observed wavelength	Functional Group	Vibrations
1	3200-3500	3302.19	OH	Streching
2	1650-1700	1652.01	C=O	Streching
3	1000-1200	1103.09	C-O	Streching
4	1300-1500	1455.55	C-H	Bending

The above FTIR spectrum indicates the functional group of standard rutin according to reference and isolated compound is approximately same. So accoding to IR spectrum rutin may present.

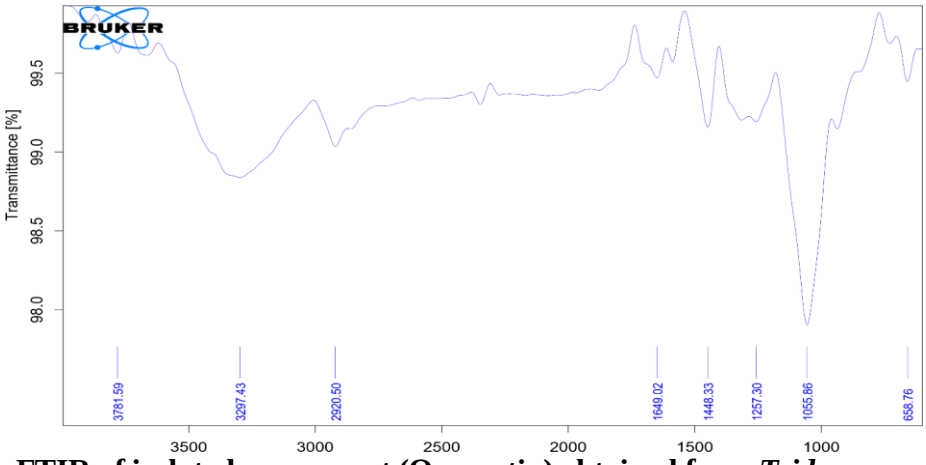


Fig. 21: FTIR of isolated component (Quercetin) obtained from *Tridax procumbens.*

Table No. 9: FTIR data of isolated component from *Tridax procumbens.*

Sr no.	Standard Wavelength	Observed wavelength	Functional Group	Vibration
1	3200-3400	3297.43	OH	Streching
2	1600-1620	1649.02	C=C	Streching
3	1000-1200	1055.86	C-O	Streching
4	1300-1500	1448.33	C-H	Bending

The above FTIR spectrum indicates the functional group of standard quercetin according to reference and isolated compound is approximately same. So accoding to IR spectrum quercetin may present.

Physical evaluation of gel

On the basis of Organoleptic properties of herbal extract i.e *Hemidesmus indicus and Tridax procumbens* following observations are shown.

Observations of organoleptic properties

Table no. 10: Organoleptic properties.

Sr. no	Test	Observations
1	Colour	Dark green
2	Odour	Characteristics
3	Greasiness	Non greasy
4	Consistency	Smooth
5	Homogeneity	Homogenous

pH Determination

It was found to be in the range of 6.62 to 7.08, kept at different storage conditions for 5 days. pH of the formulations and base kept at 8°C for 5 days did not show much change and data were significant over control (base) during one month ($p < 0.03$). Interestingly at 40°C, formulation exhibited elevated change in pH (7.08), while the others remained slightly stable during 5 days study. Data of formulation at 40°C were found to be significant in following table.

Fig. 22:- pH apparatus.

pH of the formulation at different storage condition

Table no. 11: pH of the formulation at different storage condition.

Duration	Storage condition	pH
1 day	8 ^{0}C	6.63 ± 0.22
	40^{0}C	6.91 ± 0.17
3 days	8 ^{0}C	6.87 ± 0.19
	40^{0}C	6.93 ± 0.23
5 days	8 ^{0}C	6.80 ± 0.38
	40^{0}C	7.0 ± 0.14

Spreadability

Table no. 12: Spreadibility of formulation.

Test	Observation	Result
Spreadability	6.7 gm.cm/sec	Easily spreadable

Spreadability of the formulation were studied and found to be 6.7 gm.cm/sec. the formulation were found topossess good spreadability.

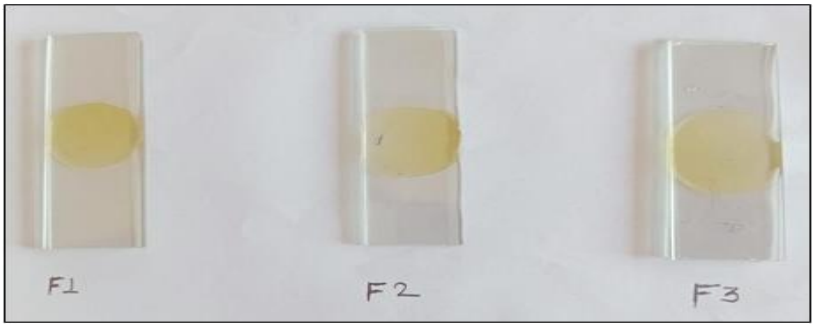


Fig. 23: Spreadability Test.

Viscosity test

Viscosity and rheological parameter of formulation were found to be 5.15 Cps. The data of viscosity information were significant temperature.

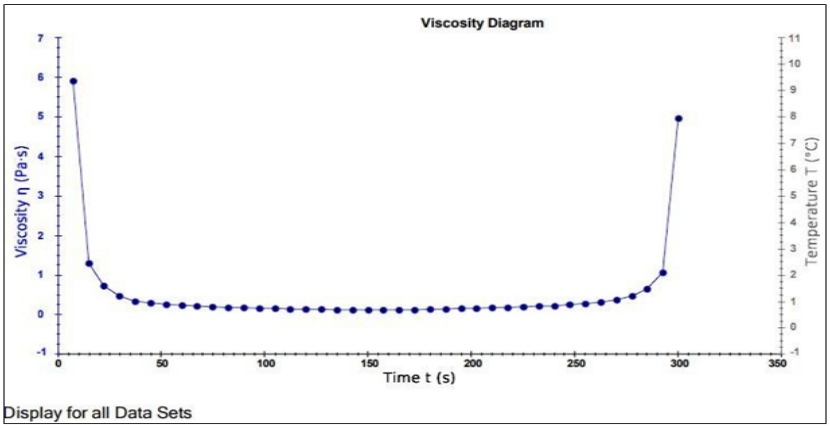


Fig. 24: Viscosity Test.

Antifungal activity

Antifungal Activity of methanolic Extract of *H. Indicus and T. Procumbens* against *candida albicans.*

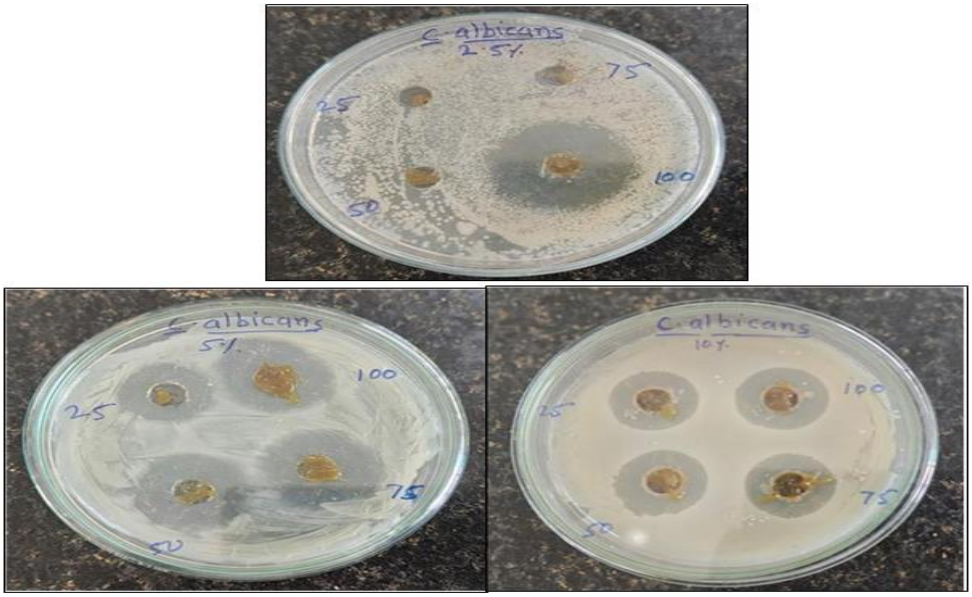
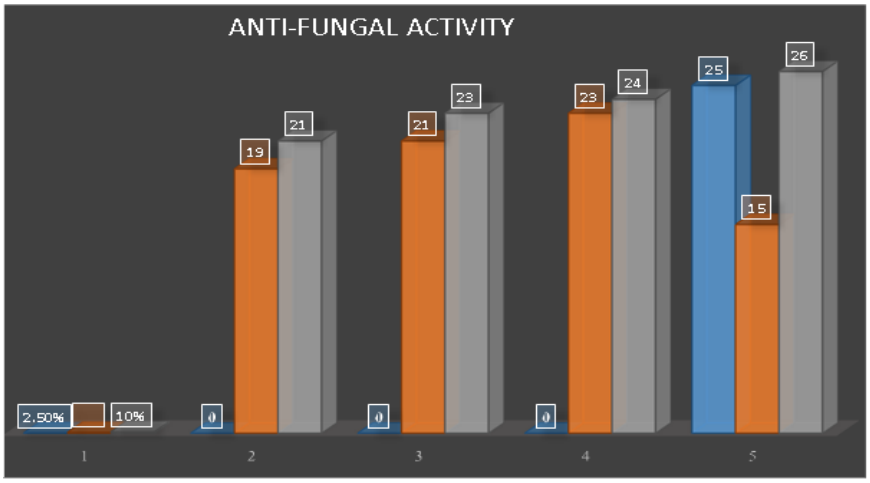


Fig. 25: Zone of inhibition of herbal formulation against candida albicans.

Table no. 13: Zone of Inhibition in mm.

Test of Organism *Candida albicans*	Zone of Inhibition in mm			
Drug Concentration	**25 µg**	**50 µg**	**75 µg**	**100 µg**
2.5%	00	00	00	25
5%	19	21	23	15
10%	21	23	24	26



Current study gives idea about antifungal activity of final formulation at different concentrations against test pathogen.

The antifungal activity of the formulated herbal extract was evaluated against Candida albicans by Agar well diffusion assay three different concentrations (2.5%, 5%, and 10%) and across four different doses (25 µg, 50 µg, 75 µg, and 100 µg). The zone of inhibition was measured in millimeters (mm) to assess the efficacy of the formulation. The results demonstrated a clear concentration-dependent and dose-dependent antifungal activity. The 2.5% formulation exhibited no activity at 25 µg, 50 µg, and 75 µg, but showed moderate inhibition (25 mm) at 100 µg.

The 5% formulation showed better activity, with inhibition zones increasing from 19 mm (25 µg) to 23 mm (75 µg), though a drop to 15 mm was observed at 100 µg, possibly due to formulation saturation or antagonistic interaction at higher dose.

The 10% formulation displayed the highest antifungal activity, with inhibition zones ranging from 21 mm to 26 mm, peaking at 100 µg. This suggests that the antifungal activity of the final formulation is positively influenced by increasing both the concentration and dose of the herbal extract, with 10% at 100 µg being the most effective combination. These findings indicate a synergistic effect of the combined herbal components in the formulation and support their potential application in antifungal therapies targeting Candida albicans.

CONCLUSION

The present investigation highlights a scientifically validated approach to developing a potent herbal antifungal formulation. Soxhlet extraction of the selected plant materials yielded significant phytoconstituents, confirmed by preliminary phytochemical screening to be rich in flavonoids. Subsequent spectral characterization using UV-Visible and IR spectroscopy confirmed the presence of key bioactive compounds, namely quercetin and rutin, both of which are well- documented in the literature for their antimicrobial efficacy and synergistic effects.

By performing UV of Isolated component of rutin of Hemidesmus indicus and Quercetin obtained from Tridax procumbens indicates the presence of Rutin & Quercetin in sample by approximately matching of λmax of std and observation. FTIR isolated components of Rutin and Quercetin obtained from Hemidesmus indicus and Tridax Procumbens repectively, shows the stretching & bending approximately it is similar in std & observations of the peak.

Pharmacological evaluation against Candida albicans revealed a marked concentration- and

dose- dependent antifungal activity, with the 2.5% extract showing the highest zone of inhibition (25 mm at 100 µg). These findings corroborate existing studies on flavonoid-based therapeutics and provide strong evidence for the antifungal potential of the formulated extract. The study not only supports the ethnopharmacological claims but also contributes to the growing body of evidence advocating plant-based antifungal agents as viable alternatives to synthetic drugs, especially in light of increasing antifungal resistance. Further in vivo studies and compound isolation are recommended to fully elucidate the therapeutic potential and safety profile of the active constituents.

REFERENCES

1. Rituraj Rajjan Singh, Somprabha Madhukar, Shruti Rathore; Formulation and Evaluation Of Herbal Gel; ISSN: 0975-4725
2. Shravani R Kakad, Pruthviraj M Rathod, Dhanashri A Dhanbhar, Monika S Kherade, Dr. Dipti B Ruikar; A Comprehensive Review on Hemidesmus indicus; February, 2024; 30: 2. ISSN-2349-7203
3. Mahima Anil Nikam, Pranali Rajendra Ner, Janhavi Anil Nikam; Review on Tridax Procumbens E-ISSN 2582-2160 Volume 6 IJFMR
4. Navkiranjeet Kaur, Aarti Bains, Ravinder Kaushik, Sanju B Dhull; A review on Antifungal Efficacy of plant Extract.doi:10.3390/nu13062055.
5. Satish V Deore; Review on medicinal and phytochemicals aspects of Hemidesmus indicus (L.) R. Br; ISSN: 2455-541X, 2021; 6, 4: 651-654.
6. Rajan. S, Shalini. R, Bharathi. C, Aruna. V, Elgin. A, Brindha. P; Pharmacognostical and Phytochemical Studies on Hemidesmus Indicus Root; ISSN: 0975 4873
7. Zafar Mehmood, Arunesh K Dikshit, Ajay Singh ; Indian Herb hemidesmus Indicus- A potential source of new antimicrobial compound; ISSIN: 2161-0444, 6(12): 734-738(2016)-734.
8. Saroj Yadav, Kamal Kishor Upadhay, Praveena R, Senthil Rajan Dharmalingam, Formulation evaluation and invitro antifungal assessment of A Topical preparation Using The Extract from Tridax Procumbens; ISSN1695-7504, 2024; 25: 1.
9. Sowmya B Jhample, Sanjivani B Gajdhane, Pramod J Kasabe; Phytochemical Screening and In vitro Antimicrobial Activity of Tridax procumbes L ISSN2454-6348. Research Journal of life scince (RJLBPCS2015)
10. Varsharani V. Ingole, Pravin C. Mhaske, Sushma R. Katade; Phytochemistry and pharmacological aspects of *Tridax procumbens* (L.): A systematic and comprehensive

review, 2022; 2, 1: 100199.

11. Ch.Mohan, S. Sridhar, B.Mohan: Antibacterial Activity and Phytochemical Screening of Hemidesmus Indicus L.Br, ISSN2320-7051.
12. Lalit Kumar1, Ruchi Verma2, In vitro evaluation of topical gel prepared using natural polymer, International journal of Drug Delivery, 2010; 2: 58-63.
13. Koka Sweta S, Pancholi Mamta, Sharma Vijay, Gayakwad Devshree, Darwhekar G N; Formulation and Evaluation of Topical Antifungal Herbal Gels Containing Hydroalcoholic Extract of Catharanthus roseus and Aloe vera; ISSN: 0975-4873
14. Atish K. Maldhure1, Nilima A. Kalambe 2, Dnyansagar D. Bhokare3; Antifungal activity of extract from the leaves of Tridax procumbens Linn; ISSN No. 2347-517X
15. Vignesh Kanna Balaji, Swapna Muthuswamy, Joshy Maducolil. Easow; Qualitative and Quantitative Analysis of Phytochemicals in Hemidesmus Indicus (L.) R. Br; ISSN 2278 – 4357
16. Aryan Mankotia, Shalu Dhiman and Jyoti Gupta; Preparation And Evaluation Of Herbal Gel: A Review; ISSN 2394-3211
17. Nabamita Basu, Sainuthana G, Rasmeen Fathima, Lohithalekha Sree, Hephzibha; Formulation, Development and Evaluation of Antifungal Herbal Gel; ISSN 0976-2779
18. Asna Badiuzzaman, Shaikh Mohammed Rehan, Amirulla Qadri; Formulation and Evaluation of Anti Fungal Herbal Gel Containing Neem And Lantana Camara Extract; ISSN: 2320-2882
19. Ajay Kumar Suryawanshi, Purnima Baghel, Sweety Lanjhiyana; A Review on The Design, Formulation, and Characterization of Effective Anti-Fungal And Anti-Microbial Poly-Herbal Formulations; ISSN: 0975-4725
20. Ms. Rashmi R Balkate1, Ms. Shinde Devata Haridas; A Research on Formulation and Evaluation of Herbal Gel; ISSN 2582-7421