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# EXPLORING MEDICINAL POTENTIAL: FORMULATION AND EVALUATION OF TRIDAX PROCUMBENS EXTRACTS IN TOPICAL FORMULATIONS

[1]Kiran R. Shanware, [2]Pranav P. Ghatte, [3]Pranali D. Bhagate, [4]N. B. Chougle, [5]V. A. Mahajan

[1]Student, [2]Student, [3]Student, [5]Principle, [6]Assistant Professor

[1]Student of Ashokrao Mane Institute of Pharmacy Ambap, Kolhapur 416112

**ABSTARACT:**

One significant source of possible medicinal chemicals is tr conventional medical care. Due to Tridax procumbens' potent inhibit the bacteria and destroy the fungi qualities, well, it is highly valued in tradtional medicine. The use of herbal medicines and interest in them has grown significantly in recent years, even in places with access to contemporary medical care. The present endeavor's objective is as to formulate and evaluate the extracts of Areca catechu and Tridax procumbens in ointment, cream, and gel formulations. The compositions made use of the methanolic extracts. Following formulation completion, the formulations' physicochemical characteristics, such as colour, smell, pH, spread ability, extrudability, consistency, diffusion analysis, solubility, and washability, were assessed.

Commonly used as a therapeutic herb, Tridax procumbens most famous for being a widespread weed. and nuisance plant found all over India. The plant's chemical composition revealed that the leaves include a variety of alkaloids, flavonoids, carotenoids, fumaric acid, and other compounds. Numerous pharmacological characteristics are present in their extract, including anti-inflammatory, hepatoprotective, immunomodulatory, antimicrobial or antibacterial, antiseptic, anti-cancerous, repellent, haemostatic, antidiabetic, anti-urolithiatic, hypotensive, antioxidant effect, and bradycardiac effects. The plant Tridax procumbens, which has long been recognised for its antibacterial properties, contains large amounts of phenolic chemicals, which are secondary metabolites.

**Keywords:** Herbal medicine, healing property, formulation, extraction, pharmacognostic.

## ❖ INTRODUCTION:

Tridax procumbens Linn., it is a topical plant that is native to tropical and subtropical regions. a member among the Asteraceae relatives. Actually, it is referred to as Tridhara or Bishalyakarani in West Bengal [1]. It is a tiny, semi-prosthetic, herbaceous creeper weed with short, hairy leaves that resemble blades that can be annual or perennial. Corolla has a yellow hue. The stem is elongated, branching, sparsely hairy, and roots at nodes. It can reach a height of 20 to 60 cm. Simple, opposite, stipulate, lanceolate, or ovate leaves are available. 4–8 cm in length, with a toothed edge, An elongated foundation, a brief petiole, as well hair regarding both surfaces. The yellow, hairy flowers have a tubular form with a capitulum inflorescence. Ray florets and disc florets are the two floral kinds found on the plant [2]. The plant is examined to check for the presence of components of phytochemistry and antioxidant attributes [3, 4, 5]. The plant has demonstrated efficacy in

treating a number of circumstances, such as the healing of wounds [6], diarrhoea [7], schizophrenia, malarial illness [8], nausea, diarrhoea, elevated BP, insulin [9], haemorrhage, and metabolic syndrome [10].

The examination of phytochemicals showed existence of carotenoids, alkaloids, tannins, saponins, and flavonoids (catechins and flavones) [11]. Several synthetic medications made by the pharmaceutical industry have occasionally led to The appearance of resilient microorganisms, which is now an important worldwide issue in the therapy for infectious illnesses. It's been demonstrated that antibacterial medications derived from plants are effective and have fewer side effects [12]. Approximately twenty-five thousand species make up the Asteraceae family, A large number of which are rich in further metabolites having physiological function [13].

The phytochemicals found in fruits and vegetables, like phenolics, pigments, and fat soluble vitamin and ascorbic acid of water soluble vitamin , are mostly responsible for these health advantages. vegetation containing one or more sets of hydroxyls attached to one or more fragrant rings produce secondary metabolites called polyphenols. Thousands of polyphenolic chemicals have been found in higher plants, including food plants. The two primary categories of plant polyphenols are flavonoids and non-flavonoids [14].

- **Scientific categorising**

**Kingdom** - Plantae,
**Subkingdom** - Tracheobionta,
**Division**- Magnoliophyta,
**Class** - Magnoliopsida,
**Subclass** - Asteridae,
**Order** - Asterales,
**Family** - Asteraceae,
**Genus** - Tridax
**Species** - Tridax procumbens [15]

- **Botanical Name: Tridax procumbens Linn.**

**Synonym**
**Bengali:** Tridhara/Bishalya Karani
**Hindi**: Khal muriya, Ghamra
**Sanskrit**: Jayanti Veda
**English**: Coat buttons, Tridax Daisy, Mexican Daisy
**Oriya**: Bishalya Karani
**Marathi**: Gaddi Chemanthi
**Tamil**: Vettukaya thalai, Thatha
**Telugu:** Gayapu aku/Palaka aku

- **Preparation of cultivate extract:**

Tridax procumbens fresh leaves were washed and patted dry. In a mixer, the 700 g of leaves were pulverised without the addition of water or any other material. Juice from 600 cc of leaves was extracted by straining the extract through muslin cloth. After that, a 300 ml filtrate
was centrifuged at 1000 rpm for 15 minutes using an Eppendorf centrifuge. A 225 ml supernatant was extracted from this. For fifteen to twenty minutes, the supernatant solution was frozen using dry ice and acetone. After that, the frozen compound was stored in a freeze dryer for 24 hours under vacuum and at -47 0 C for lyophilization [16, 17].. Following full drying, a about 5 g water soluble leaf extract powder was produced. By dissolving the powdered substance made from the extract of Tridax procumbens leaves in normal saline, it was given orally at various doses. Two drops of Tween 80 and 0.5% (w/v) methyl cellulose were added to a suspension of tramadol hydrochloride. Diclofenac sodium was given in a standard saline solution. [18–19]

**fig 1 preparation of cultivate extract.**

- **GOALS:**

-To extract the Tridax procumbens leaves using an appropriate solvent (ethanol). The use appropriate chemical separation to recover the chemical ingredient from the crude extract of Tridax procumbens leaves.
- To use spectroscopic and chemical analysis to analyse the chemical group present in the crude extract of Tridax procumbens leaves in order to gain a general understanding of the compound present in the extract.

- **BOTANICAL MORPHOLOGY [20,21]**

**A: LEAVES:**

**fig 2 leaves of tridax procumbens**

The leaves are lanceolate, simple, ovate, opposite, exstipulate, and irregularly toothed, with an overall arrowhead shape. They measure 3-7 cm. basal lead with a wedge form, small petioles, and hairy surfaces on both sides.

**B: STEM:**

**fig 3 stem of tridax procumbens**

The plant stem is rooted at nodes and ascends to a height of 30 to 50 cm. It is sparsely branched.

**C: FLOWER:**

**fig 4 flower of tridax procumbens**

- The plant has daisy-like blossoms.
- The flower has serrated ray florets and is tubular, yellow, and has a white or yellow core.
- capitulum of inflorescence. It has two types of flowers.
- Ray dies florets and leaves behind basal placentation.

**D: FRUIT:**

**fig 5 fruit of tridax procumbens**

- Fruit is a firm achene with feathery texture and stiff hairs covering it.
- It features a white papapus-like plume at one end.

**E: SEED:**

**fig 6 seed of tridax procumbens**

Produlous embryo endosperm is lacking from plant seeds.

❖ **Morphology [22,23,24,25]**

**table 1 morphology of tridax procumbens plant**

Tridax procumbens is a herbaceous perennial plant with a spreading basal section that can reach a height of 40 cm or more.

| | |
|---|---|
| Root | branching, suberect, pilose (thickly hairy), creeping at the base, or trailing above. |
| Leave | Acute apex, simple, opposite, elliptic-rhomboid, ovate-lanceolate, or elliptic-rhomboid, cuneate base, clearly hispid, and serrated to the coarsely dentate. |
| Flowers/Inflorescence | Heads are actinomorphic, pentamerous, and bisexual.<br>single, 1.2–1.5 cm across, with several yellowish tubular–campanulate disc floret involucres per head. The length of the poduncle is 10–30 cm. |
| Calyx | reduced to pappus, characterised by scales. |
| Fruit | Stiff hairs covering a hard achene fruit at one end. Its white pappus is fluffy and resembles a plume. |
| Seed | There is no endosperm in the plant seeds because the embryo is pendulous... |
| Root | Taproot framework |

❖ **CHEMICAL CONSTITUENT**:

Numerous investigations have demonstrated that the plant contains a variety of phytochemical substances. This medicinal plant contains alkaloids, carotenoids, saponins, flavonoids, and tannins, according to the results of the phytochemical screening. Tridax procumbens has a high concentration of calcium, potassium, and salt, according to its proximate properties [26]. The plant exhibits a variety of chemical components, including pinostrobin, dihydroechioidinin, tectochrysin, 2,6-dihydroxyacetophenone, 2-O-β-D-glucopyranoside, and echioidinin. 5-glucoside, glucoside methyl salicylate, Skullcapflavone I 2-methyl ether, 5,7,8-trimethoxyflavone, androechin, tectochrysin, 5,7,2-trimethoxyflavone, echioidin, 5,7-dimethoxyflavone, andrographidine in [27]

Quercetagetin-3,6,4'-trimethoxy-7-O-neohesperidoside (flavanol diglycoside), 1,2-dihydrodendroarboreol B (a polyacetylene), and (3S,5R,6S,7E) are examples of phytochemicals. From the plant's ethanolic extract, -three-tetradecanoate-5,6-epoxy-β-ionone (an ionone derivative) has been clearly identified [28]. It has been predicted that Tridax procumbens has a general chlorophyll content of 1.424±0.017 mg/g and a general carotenoids content of 0.724±0.007 mg/g [29]. The plant has high concentrations of tannins, phytosterols, flavonoids, hydroxycinnamates, and alkaloids [30].
Numerous chemicals have been isolated from plant components, including betulinic acid, esculetin, puerarin, oleanolic acid, and two novel flavones designated 6,8, three′-trihydroxy-three,7,4′-trimethoxyflavone and 8, three′-dihydroxy-three,7,4′- trimethoxy-6-O-β-D-glucopyranosyl flavone [31]. The α-glucosidase inhibitory property was demonstrated by oleanolic acid obtained from Tridax procumbens [32].

A novel bisbithiophene called tri-bisbithiophene, together with terpenoids such as β-amyrenone, oleanolic acids, taraxasteryl acetate, and lupeol, were isolated from a hexane extract of ethyl acetate-soluble Tridax procumbens portion [33]. Tridax procumbens Linn leaves were used to purify two water soluble polysaccharide fractions: WSTP-IA (~1: three molar proportions of l-Araf and d-Galp) and WSTP-IB (more effective d-Galp because the primary sugar component) [34]. The hydro-distilled essential oil of Tridax procumbens contains the following important compounds: 1,8-cineole, p-cymen-7-ol, Biformene, Trans-(α)-caryophyllene, and Dibutyl phthalate as the main compounds; 9,12,15-octadecatrienoic acid, Z-α-trans Bergamotol, α- Elemene, 2-aα-pinene, Tricosane, α-Selinine, α-Amyrin, Caryophyllene oxide, α-humulene, Eicosane, Quercetin 7, three',4'-trimethyl, and tau-cadinol as the minor compounds.[35].

### ❖ TRADITIONAL USES

Tridax procumbens is a common weed and blooming plant with a variety of therapeutic uses. It has long been used in India as a wound healing agent, insect repellant, anticoagulant, and antimicrobial. Additionally, it is used to treat blisters and boils. This herbaceous plant is commonly used as a folk remedy for hair and ulcers. In ethnomedical practises, its leaf decoctions were used to cure infectious skin ailments. Because the plant decoctions have hepato-protective properties, it is a well-known ayurvedic treatment for liver problems. In addition, the extracts are utilised to treat heartburn and gastritis [36]. It is frequently used to halt bleeding from cuts, bruises, and wounds in the healing process. The herb is also used to treat severe diarrhoea and dysentery, as well as excessive blood pressure and blood glucose levels. [37,38,39].

It helps to stimulate hair development and can be used to stop hair loss. The herb is also used to treat respiratory conditions. It possesses strong insect repellant and immunomodulatory properties [40]. The plant's leaves are used as a conjunctivitis cure by tribal peoples and rural medical practitioners in West Africa and the tropical regions of the world [41]. The ethnic system also employed this medicinal herb to treat liver problems and jaundice [42]. Kidney stone illnesses were also treated with Tridax procumbens ethanol decoctions [43].

### ❖ EXTRACTION:

The plant material can be extracted by drying the specified plant in the shade and then grinding it into a fine powder. After that, the dried powdered material is percolated in a Soxhlet extractor for 48 hours using the polar solvent hexane, then with the non-polar solvent hexane.

- **Procedure of Extraction**

1.124 grammes. Tridax procumbens powder is added to a Soxhlet extractor and kept at a constant level.

2. A 5000 ml round-bottom flask is filled with one litre of ethanol. On moveable elements, hoover grease is applied to reduce friction.
3. To stop connectors from leaking, cotton and aluminium foil are also used to cover them.
4. After the Soxhlet extractor is set, the heating mantle provides power and keeps the temperature at 60°C.
5. After that, a chiller is linked to a Soxhlet extractor to give chilling.
6. For 48 hours, same process is carried out again.
7. Distillation is then used to separate the ethanol extract from the crude extraction.
8. With the use of ethanol, we discovered the 22.5 gramme. of crude leaves.
9. Add hexane to the crude, strain off the soluble material, and use TLC to confirm. The entire mass of Ten grammes of crude hexane separated [44].

### ❖ IDENTIFICATION TEST:

Plant preliminary phytochemical screening: Standard procedures were used to extract different solvents and check if a plant's phytochemical profile contained any bioactive compounds that were slightly modified [45]. One millilitre of plant extract should be mixed with a few drops of Mayer's reagent to check for alkaloids. Alkaloids were present in precipitate that turned swiftly from white to yellowish in hue [46]. Alkaloids are precipitated from neutral or slightly acidic solutions using Mayer's reagent [47].

- **To test for terpenoids and steroids, :** 4 ml of extracts were treated with 0.5 ml of chloroform and 0.5 ml of acetic anhydride to check for terpenoids and steroids. Then, concentrated sulphur dioxide was added gradually. Steroid Solution 2 exhibits a green-blue tint, but Terpenoid Solution displays a reddish-violet colour. Rh. Flavonoid and flavone test: 1.5 ml of a 50% methanol solution was added to 4 ml of extracts, the solution was heated, metal magnesium was added, and finally, 5–6 drops of strong hydrochloric acid were added. The hue of the flavonoid solution is red, while the avones solution is orange [46].
- **Test for tannins:** 0.5 ml of extract solution was mixed with 1 ml of distilled water, and then A few of drops of the ferric chloride solution were added. Catecholic tannin solution displays green and black hue, while gallic tannin solution displays blue colour.
- **Test for reducing sugars:** To 0.5 ml of extract solution, add 1 ml of distilled water. Then, add Fehling's solution in five to eight drops, A and B, at room temperature, respectively. When sugar is reduced, a red-brick precipitate form.

- **Test for carbohydrates (Molish's test):** Two drops of α-naphthol solution were added to one millilitre of extract. The tube was then gently tilted, and dropwise concentrated H SO was added using droper 2 4 along the side to the tube. Violet hue indicates the presence of carbohydrates where two liquids converge.
- **Test for glycosides:** A small amount of FeCl is added after One millilitre of glacial acetic acid and one millilitre of extract are combined. A brown colour ring that appears at the top denotes the presence of three glycosides.
- **Test for saponins (foam test):** In a test tube, 1 millilitre of the extract was mixed with 2 millilitres of distilled water and agitated for a few minutes. For ten minutes, a 1-centimetre layer of foam suggests the presence of saponins.
- **Test for phenols (Ferric chloride Test):** After dissolving 1 millilitre of extract in 1 millilitre of ethanol or distilled water and a few drops of ferric chloride solution are added. The phenolic solution has hues of red, blue, green, and purple.
- **Test for proteins (Xanthoproteic test):** There was one millilitre of extract added. two to six drops of concentrated HNO Alkali was used to neutralise solution 3. Protein solutions have an orange or yellow tint.

❖ **FORMULATION:**

**table 2 formulation of herbal ointment**

| Sr no | Name of ingredients | F1 (gm) | F2 (gm) | F3 (gm) | F4 (gm) |
|---|---|---|---|---|---|
| 1. | Wool Fat | 1 | 1.5 | 1.5 | 1.5 |
| 2. | Cetostearyl Alcohol | 1 | 2 | 3 | 4 |
| 3. | Stearic Acid | 1 | 2 | 3 | 4 |
| 4. | White Soft Paraffin | 4.5 | 4 | 4 | 4 |
| 5. | Tridax Procumbens Extract | 1 | 1 | 1 | 1 |
| 6. | Neem Extract | 1 | 1 | 1 | 1 |
| 7. | Ointment base | 7.5 | 9.5 | 11.5 | 13.5 |

**A) THE HERBAL OINTMENT PREP ROUTINE [48, 49].**

1. Using wool fat, white soft paraffin, cetostearyl alcohol, and steric acid, the ointment base was first made. It was then precisely weighed and put in an evaporating dish over a water bath. After the melting, the ointment base is cooled, and the combination is gently mixed to create a homogenous mixture.
2. To produce a smooth paste with twice or three times the weight of the base, precisely weigh the Neem and Tridax procumbence extract. Then, add more base gradually until the ointment is homogenous. Finally, the mixture is transferred into an appropriate container.

**a) GEL:**

The gel was prepared by using Carbopol 940 (1%), propylene glycol, ethanol, propyl paraben, methyl paraben EDTA di-sodium, triethanolamine and distilled water in quantity to prepare 100gm of gel. The quantity of distilled water required for the formulation is divided into two parts. In one part the exact amount of extract was dissolved and to this calculated amount of ethanol and propylene glycol was added and in other part Carbopol 940 was dissolved and to that solution propyl paraben, methyl paraben and EDTA disodium was added. Both of that solutions were mixed in beaker and triethanolamine was drop wised added in mixture to obtained gel consistency [50]

**Table 3 formulation of gel**

| Formulation Code | Carbopol-940 (gm) | Extract (gm) | Propylene glycol (ml) | Ethanol (ml) | Methyl Paraben (gm) | Propyl Paraben (gm) | EDTA (gm) | Distilled water up to(ml) | Triethanolamine (ml) |
|---|---|---|---|---|---|---|---|---|---|
| F1 | 4 | 9 | 4 | 3 | 0.2 | 0.02 | 0.03 | 100 | q. s |
| F2 | 1 | 9 | 2.5 | 3 | 0.2 | 0.02 | 0.03 | 100 | q. s |
| F3 | 2.5 | 9 | 1 | 3 | 0.2 | 0.02 | 0.03 | 100 | q. s |
| F4 | 4 | 9 | 2.5 | 3 | 0.2 | 0.02 | 0.03 | 100 | q. s |
| F5 | 2.5 | 9 | 2.5 | 3 | 0.2 | 0.02 | 0.03 | 100 | q. s |
| F6 | 2.5 | 9 | 4 | 3 | 0.2 | 0.02 | 0.03 | 100 | q.s |
| F7 | 4 | 9 | 1 | 3 | 0.2 | 0.02 | 0.03 | 100 | q. s |
| F8 | 1 | 9 | 4 | 3 | 0.2 | 0.02 | 0.03 | 100 | q. s |
| F9 | 1 | 9 | 1 | 3 | 0.2 | 0.02 | 0.03 | 100 | q.s |

**b) SOAPS:**

The glassware is sterilised using the dry heat method. Glycerine soap base weighing 100g was melted. Alcohol-free coconut oil and sodium hydroxide are used to make the glycerine soap base. Aloe vera gel, coconut oil, honey, and plant extract (as per the formulation design) were combined with vitamin E oil in a different beaker until everything was fully dissolved. For scent, add 1ml of Tulasi essential oil to the mixture. Lastly, the melted soap base is mixed with the plant extract mixture. Poured into moulds, this mixture is left to solidify at room temperature.

**Table 4 formulation of soap**

| Ingredients | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Plant extract | 0.5 g | 1.0 g | 1.5 g | 2.0 g |
| Soap base | 100 g | 100 g | 100 g | 100 g |
| Vitamin E | 400 mg | 400 mg | 400 mg | 400 mg |
| Tulasi oil | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml |
| Aloe vera gel | - | 10 g | - | - |
| Honey | 5 g | - | - | - |
| Coconut oil | - | - | - | 10 g |
| Total weight | 105.9 g | 111.4 g | 101.9 g | 112.4 g |

**(-) indicates that the component is not included in the recipe.)**

## ❖ EVALUATION TEST:

### a) OINTMENT:

**1) PH:** A digital PH metre was used to measure the PH of the produced herbal ointment. Ten millilitres of distilled water were used to produce the ointment solution, which was then left for five minutes.
**2) Colour and Odour:** Visual inspection was used to assess physical characteristics including colour and smell.
**3) Consistency:** Nothing gaudy or avaricious is shown.
**4) Spread ability:** By sandwiching an extra sample between two slides that had been crushed to a consistent thickness by applying a specific weight for a specific amount of time, the spread ability was ascertained. Spread ability was calculated as the amount of time needed to separate the two slides. Improved spread ability is the outcome of taking less time to separate two slides.
The formula used to calculate spread ability was as follows:

$$S = M \times L / T$$

Where, S- spread ability.
M- weight of sample in gramT
Time taken in seconds.
**5) Extrudability**: A collapsible tube container was filled with the formation. Extrudability was measured as the weight of formulation needed to extrude 0.5 cm of ointment ribbon in 10 seconds.
**6) Loss on drying:** The formulation was dried at 105°C in a petri dish set over an oil bath to ascertain the amount of loss.
**7) Solubility**: Miscible with ether, alcohol, and chloroform; soluble in boiling water
**8) Washability**: The skin was treated with the formulation, followed by a gentle water wash and inspection.
**9) Non-irritancy:** A human subject had the produced formulation applied to their skin, and the results were monitored.
**10) Stability research:** At 370 C for several physicochemical parameters, physical stability study tests of the formulation were conducted on the first day, after three months, and after six months. specifications. For six months, the formulation was determined to be physically stable at several physicochemical parameters.
**11) Viscosity**: A Brook field viscometer was used to measure the produced ointment's viscosity.

### b) GEL:

Physical characteristics like look and colour were assessed. Homogeneity: After the gels were placed in the container and examined visually for appearance and the existence of any aggregates, all generated gels were checked for homogeneity.

**i)pH:**

A digital pH metre was used to measure the pH of several gel compositions. After precisely weighing 2.5 grammes of gel, it was mixed with 25 millilitres of distilled water and kept for two hours. Three measurements of the pH were made for each formulation, and the average results are shown. A pH metre was used to determine the dispersions' pH [51].

**ii)Spread ability:**

The device, which consists of a wooden block supplied by a pulley at one end, was used to measure spread ability. This approach tested spread ability based on the gels' slide and drag properties. On this ground slide, an excess of the gel under investigation (about 2 g) was added. After that, the gel was positioned with the hook between this glass slide and another one that had the same dimensions as a fixed ground slide. For five minutes, a one kilogramme weight was applied to the top of each slide in order to force out air and create a consistent layer of gel between the slides. The excess gel was removed by scraping off the edges.
After then, a 50 g pull was applied to the top plate. Determine how long it takes the top slide to travel 6.5 cm (measured in seconds) using a piece of string fastened to the hook. Better spread ability is indicated by a shorter interval [52].
Spread ability was determined by applying the subsequent formula:

$$S = M \times L / T \qquad \text{……Eq1}$$

Where, S = Spread ability,
M = Weight in the pan (tied to the upper slide),
L = Length moved by the glass slide and
T = Time (in sec.) taken to separate the slide completely each other.

**iii)Microbial growth:**

A microbial growth investigation was conducted using nutrient agar medium. Using this technique, gel samples were aseptically transferred in a cross pattern onto blank and sample Petri plates. The proliferation of microorganisms noted [53].

**iv)Viscosity:**

The viscosity of the herbal gel was measured at 5, 10, 20, 30, and 50 rpm using a Brookfield rotating viscometer with spindle number 64. After the sample had reached equilibrium after two minutes, each reading was obtained. Three iterations were conducted in the viscosity determination of the samples.

**v)Antimicrobial activity:**

Using the ditch plate method, the produced gel's antimicrobial properties were investigated. It is a method mostly utilised in semisolid formulation for assessing a compound's bacteriostatic and fungistatic activities. Standard protocol was followed in the preparation and sterilisation of agar plates. A test formulation was used to fill a trench that was created in the middle of the agar plate. From the ditch to the plate's edge, the prepared culture loops were streaked at a straight angle across the agar. The bacterial growth was monitored, and the degree of inhibition was evaluated following an 18–24-hour incubation period at 250 C

**c)SOAPS:**

**1. Physical characteristics of the soap formulation were examined**: colour, clarity, and odour were all assessed visually on a white background. We looked at these characteristics in each of the four formulations.

**2. To find the pH:** dissolve 5 grammes of soap in 100 millilitres of water. Using a digital pH metre (Systronics Digital pH metre MK VI), the soap solution's pH was measured. Four formulas' pH values were ascertained independently.

**3.Determination of percentage free alkali:**

A digital weighing balance (Essae Weighing balance, model DS-852G) was used to weigh 10g of sample soap. The sample soap was then placed in a beaker, 150 ml of filtered water was added, and the mixture was heated for 30 minutes under reflux in a water bath (SISCO water bath). In a beaker, the capacity was increased to 250 ml. Phenolphthalein indicator (1 millilitre) was added. It was quickly titrated with 0.1 M HCl until the mixture became colourless. 54]

**4. Determination of foam height:**

A 0.5 g sample of soap was dissolved in 25 ml of distilled water. It is transmitted into a 100 ml measuring cylinder and volume was made up to 50 ml with water. We administered 25 strokes. It can remain in place until the water content is measured up to 50 millilitres. It was measured how high the foam was above the aqueous volume [55].

**5. Determination of foam retention:**

A solution of 1% soap was made. A 100 ml graduated measuring cylinder was filled with 25 ml of 1% soap solution. Ten times, the cylinder was covered and shaken. The amount of time it took for the foam to vanish was noted [55].

**6. Determination of alcohol insoluble matter:**

The majority of alkaline salts, including talc, carbonates, borates, silicates, and phosphates, as well as sulphates and starch, which are insoluble in alcohol under test conditions, are included in the category of alcohol insoluble materials. 50 ml of warm ethanol was added to a conical flask containing 5g of soap sample, and the mixture was agitated quickly until the sample was completely dissolved. After passing the mixture through tared filter paper and 20 millilitres of warm ethanol, it was dried for an hour at 1050 degrees Celsius. It was noted how much the dry paper weighed. [54].

$$\% \textbf{ Alcohol insoluble matter} = \frac{\textbf{Weight of residue} \times \textbf{100}}{\textbf{Weight of sample } (\textbf{1})}$$

**7. Moisture content:**

The percentage of water in the soap can be estimated using the moisture content. Five grammes of soap were weighed and marked as wet weight or starting weight in order to measure the moisture content. A sample was dried for an hour at 100 to 1150 degrees Celsius using a hot air oven. After cooling, the sample was weighed. This weight is noted as the sample's dry weight. [54].

The following formula was used to calculate the moisture content.

$$\% \textbf{ moisture content} = \frac{\textbf{Initial} - \textbf{Final weight} \,.\, \textbf{100}}{}$$

❖ **BIOLOGICAL PROPERTY:**

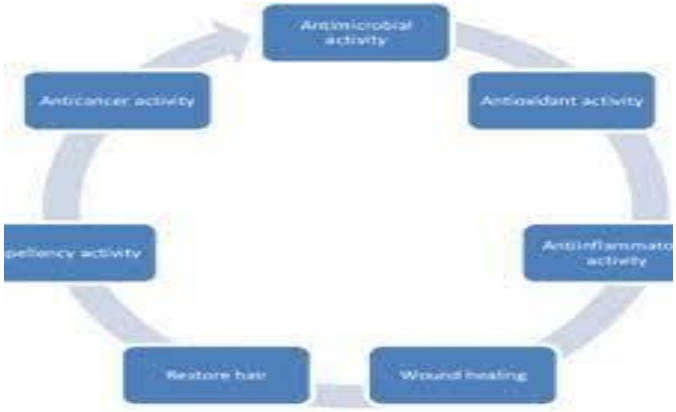


**fig 7 biological property**

Numerous solvent extracts of Tridax procumbens have been studied; these extracts have demonstrated various biological activities in animal models and have been found to possess anti-inflammatory, hepato-protective, immune-modulatory, wound-healing, anti-microbial, anti-malarial, anti-cancer, blood coagulation, repellency, anti-diabetic, anti-lithiatic, anti-obesity, anti-hyperglycemic, analgesic, hemostatic, and hypotensive qualities. Here's how these bioactivities are explained:

- **Antioxidant Activity:**

The stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was used to test the radical scavenging or hydrogen-donating capacity of the Tridax procumbens fractions and ascorbic acid [56]. The concentration (mg/ml) of the methanol extract fractions that signals the generation of DPPH radicals by 50% is known as the IC50, and it is used to express the antioxidant activity of the fractions [57].

- **: Anti-bacterial Activity**

The entire plant parts of Tridax procumbens have been shown to exhibit anti-microbial efficacy against a variety of bacterial species in a previous study. To extract juice from a whole plant, squeeze it between your palms and apply it twice a day for four to five days to wounds and cuts. With the aid of the disc diffusion experiment, the extract of the entire plant demonstrated anti-microbial properties exclusively against Pseudomonas aeruginosa. Two gram-positive strains of Bacillus subtilis and Staphylococcus aureus and two gram-negative strains of Escherichia coli and Pseudomonas aeruginosa were among the four types of bacteria used in the test [58]. This action was only clearly demonstrated by ethanol extract against strains of Pseudomonas aeruginosa. Significant sensitivity to extracts was demonstrated by multidrug-resistant nosocomial strains of Pseudomonas that were isolated from bloodstream infections, urinary tract infections, and pneumonia linked with ventilators. This study demonstrated the effectiveness of Tridax procumbens as a source of formulations for the treatment of nosocomial infections brought on by Pseudomonas aeruginosa and as an anti-pseudomonal drug [59].

- **Wound Healing Activity**

The plant decoction's ability to cure wounds is attributed to a complex interplay between plasma-derived proteins, extracellular matrix, controlled angiogenesis, and epidermal and dermal cells, all of which are regulated by growth factors and cytokines [60]. Although not as much as whole plant decoctions, waterleaf decoctions were also successfully raising lysyl oxidase. This plant's leaf extract has been shown to accelerate wound healing in both immunocompromised and healthy rats. Because of the rise in glycosaminoglycan content, the plant can raise the levels of lysyl oxidase, protein, and nucleic acid in the granulation tissue [61].

- **Anti-fungal Activity:**

To ascertain the antifungal efficacy of the plant decoctions, the disc diffusion method was applied to two fungus strains, Aspergillus flavus and Aspergillus Niger, in a research study. Minimum fungicidal and inhibitory concentrations were used to measure total activity. The decoction of flavonoids had the maximum level of

activity against Aspergillus Niger, but the decoction of alkaloids demonstrated no action against either of the test fungus [62].

- **Anti-malarial Activity:**

The ethanol and water infusions have anti-plasmodial qualities that combat Plasmodium falciparum, a parasite resistant to chloroquine. Further animal toxicity studies on the plant are necessary, however the decoctions exhibit modest toxicities to human red blood cells [63].

- **Anti-cancer Activity:**

Using the MTT assay, the cytotoxicity of the plant-derived compounds was assessed against a human lung cancer cell line. The substance displayed a 90% decrease in cell viability. The substance is lupeol, according to the results of the NMR, MS, and IR spectra. The evaluation of Lupeol's anti-cancer capability against human lung cancer cell line has been conducted by many methods, including clonogenic survival determination, cell cycle control, cell-based assay for COX-2 activity inhibition, and DNA fragmentation. According to the research, the luteol molecule at a concentration of 320 µg/ml shown strong anti-cancer action [64].

- **Blood Coagulation and Haemostatic Activity**:

Because of the strong blood coagulation activity observed in leaf decoctions, water may be employed as a powerful hemostatic agent [65]. Since the ethanol extract shortens the clotting time in the blood samples from all the studies, the haemostatic property of the plant's leaves of the various solvent extracts was ascertained in vitro using Lee-White's method [66].

- **Repellency Activity:**

In one study, essential oils were isolated from leaves using the steam distillation method, and their ability to resist the malaria parasite Anopheles Stephens locally in mosquito cages was examined [67,68]. Every essential oil was examined in three different concentrations. The plant's essential oils demonstrated a discernible repellent effect [69].

- **Anti-inflammatory Activity:**

Procumbens tridax has strong anti-inflammatory qualities. The gain in weight indicates that the plant decoction's anti-inflammatory effect might be the result of a corticotropic influence [70]. It was discovered that the plant's active portion, ethyl acetate fraction, contained moderately polar natural chemicals, such as flavonoids and alkaloids. These bioactive substances have been used to combat reactive oxidant species, which have been linked to the aetiology of infl0.ammation and associated diseases [71].

- **Hepato-protective Activity:**

The hepatoprotective characteristic of leaves significantly reduced the hepatocellular injury caused by D-galactosamine and lipopolysaccharide. Because both substances have the potential to harm liver cells, it has been suggested that they are hepatotoxic. D-galactosamine-induced multifocal necrosis and the lesion caused by viral hepatitis in humans are comparable. Within eight hours of treatment, this amino sugar produces hepatitis due to its toxicity, which also inhibits transcription and indirectly hepatic protein synthesis [72].

- **Immuno-modulatory Activity:**

In an animal model, ethanol decoctions of the plant's leaves have demonstrated immunomodulatory effects [73]. Increased levels of hemo-agglutination antibodies were also seen in conjunction with immune response stimulation [74].

- **Anti-diabetic Activity:**

Water and alcoholic decoctions of leaves showed a significant decrease in the blood glucose level in the animal model. The oral administration of acute and sub-chronic doses of 50% methanol decoctions significantly reduces fasting blood glucose levels in diabetic rats. The plant extracts have been widely used in various herbal drugs and is also reported to possess the activities of lowering blood glucose levels. The oral administration of water, alcoholic and petroleum ether decoctions of the leaves on animals at a dose level of 200 mg/kg of body weight orally administered for seven days. Among these water and alcoholic decoctions significantly reduces

the blood glucose level in the animal model and petroleum ether extract exhibits a feeble anti-diabetic effect [75].

- **Anti-lithiatic Activity:**

Ethanol extract of the plant was also used for treating kidney stone disorders. It showed activity against 0.75% v/v ethylene glycol and 2% w/v ammonium chloride induced calcium oxalate urolithiasis, and hyperoxaluria induced oxidative stress in animal models. Treatment with the decoctions of the plant was able to reduce callogenesis induced urinary excretion and renal deposition of calcium oxalate and resultant lipid peroxidation, indicating anti-urolithiasis and antioxidant effects [76,77].

- **Anti-obesity Activity** :

- In a research investigation, the animal receiving treatment with the plant decoctions showed a significant reduction in total cholesterol, triglycerides, total protein, free fatty acids and elevation of high-density lipoprotein cholesterol levels [78].

- **Analgesic Activity:**

Lyophilized decoctions of the plant were found to be potential analgesic [79]. Hypotensive Activity The cardiovascular effect was obtained from the water decoctions of leaves were investigated on anesthetized animals. The water decoctions can cause significant dose-dependent decreases in the mean arterial blood pressure. The higher dose leads to a significant reduction in heart rate whereas lower dose did not cause any changes in the same [79]

## ❖ CHROMATOGRAPHY:

### A) Gas Chromatographic–Mass Spectroscopic Analysis of the Phenolic Compounds in Tridax procumbens

The fractions were subjected to GC–MS analysis in accordance with the modified Al-Owaisi et al. [80] technique. A 100-ppm stock solution was made. One milligramme of pure crystalline gallic acid powdered standard was dissolved in ten millilitres of ethanol and methanol at an identical ratio (1:1) to create a stock solution of 100 mg/L. Next, using the stock solution, 4-point serial dilution calibration standards (1, 2, 5, 10 mg/L) were made. Agilent Technologies' 7820A gas chromatograph, coupled with its 5975C inert mass spectrometer (which has a triple axis detector and an electron-impact source), was used to perform the GC–MS analysis. Prior to calibration, m/z 69, 219502, and other ideal and sensitive settings of the instrument were checked for abundance utilising the Mass Spectrometer's auto-tuning to perfluorotributylamine (PFTBA) based on pre-established parameters. A 100 mg/L injection of gallic acid was used.

5% phenylmethyl siloxane packed into an HP-5 capillary column (30 m length × 0.32 mm diameter × 0.25 μm film thickness) used as the stationary phase for the compound separation process. With an initial nominal pressure of 0.30 psi, an average velocity of 40.10 cm/s, and a constant flow rate of 1.21 mL/min, helium was employed as the carrier gas. At 250 °C, 1 μL of the sample solution was injected. At 0.75 minutes, the purge flow to the split vent was 15 mL/min, for a total flow of 16.25 mL/min. Initially set for 50 °C (6 min), the oven was raised to 280 °C (5 min) at a rate of 5 °C per minute.

With a three-minute solvent delay, the run took 57 minutes. With an ion source temperature of 230 °C, a quadruple temperature of 150 °C, and a transfer line temperature of 280 °C, the mass spectrometer was run in electron-impact ionisation mode at 70 eV. To acquire abundant and present fragment ions, the entire scan-mode spectrum of the samples (scanning from m/z 45 to 550 amu at 2.0 s/scan rate) was performed after calibration. The mass spectra were compared to reference values found in mass spectrum libraries. The calibration curve yielded the amounts of the identified and characterised chemicals [80].

### B) Detection of Quercetin by High Performance Thin Layer Liquid Chromatography (HPTLC)

The pharmcognostical databases indicate that the flavonoid quercetin has both antidiabetic and analgesic properties. Therefore, we used the HPTLC method to identify quercetin in our Tridax procumbens leaves extract (TPLE), and the spectrum compares the identification of quercetin in the TPLE with standard quercetin.
**a) Creation of Quercetin standard solution:** 2 mg of precisely weighed Quercetin were transferred to a 100 ml volumetric flask and dissolved in 50 ml methanol to create a stock solution of standard Quercetin (20 μg/ml). After that, it was sonicated for ten minutes, and methanol was added to bring the total volume of the solutions to 100 millilitres to create stock solutions with 20 μg/ml.

**b) Sample solution preparation:** 250 mg of dried Tridax procumbens leaf extract, precisely weighed, was transferred to a 100 ml volumetric flask and dissolved in 80 ml of methanol. The contents of the flask were then filtered using Whatman No. 1 filter paper after it had been sonicated for 10 minutes. To obtain a stock solution of 2.50 mg/ml, the final volume of the solution was increased to 100 ml using methanol.

**c) Chromatographic conditions and instrumentation:** 20 cm x 10 cm aluminum-backed plates covered with silica gel 60 F 254 were used for HPTLC. Using a Camag (Muttenz, Switzerland) Lino mat V sample applicator fitted with a 100 µl Hamilton syringe, the standard solution of quercetin and the sample solution were applied to the plates as bands 8.0 mm wide, 30 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate. Ascending development to a distance of 80 mm was carried out in a Camag glass twin-through chamber that had been previously saturated with mobile phase vapour for 20 minutes, at room temperature (28 ± 2 ° C), using toluene: ethyl acetate: formic acid, 5:4:0.2 (v/v/v) as the mobile phase. Following development, the plates were dried using a hair drier and scanned using a Camag TLC scanner equipped with a Deuterium light at 380 nm [81,82].

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