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"PREPARATION & EVALUATION OF GEL OF TRIDAX PROCUMBEN L. & COMPARATIVE STUDY OF TRIDAX PROCUMBEN L. AND GEL FORMULATION "

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Introduction **:-**

- Gels are defined as semi rigid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase. The word "gel" is derived from "gelatin," and both "gel" and "jelly" can be drawn hack to the Latin Gelu for "frost" and gel are, meaning "freeze" or congeal." This origin indicates the essential idea of a liquid setting to a solid-like material that does not flow but is elastic and retains some liquid characteristics. The USP defines gels (sometimes called jellies) as semisolid systems containing either suspensions made up of small inorganic particles, or large organic molecules interpenetrated by a liquid. Where the gel mass contains a network of small separate particles, the gel is classified as a two-phase system. In a two-phase system, if the particle size of the dispersed phase is relatively large, the gel mass is sometimes called asa magma. Single-phase gels consist of organic macromolecules uniformly circulated throughout a liquid in such a way that no apparent boundaries occur between the dispersed macromolecules and the liquid. However, the formation of some polymer gels is irreversible because their chains are covalently bonded. The three-dimensional networks formed in two-phase gels and jellies are formed by several inorganic colloidal clays. Gels are generally considered to be more rigid than jellies because gels contain more covalent crosslinks, a higher density of physical bonds, or simply less liquid. Gel-forming polymers produce materials that span a range of rigidities, beginning with a sol and increasing in rigidity to a mucilage, jelly, gel, and hydrogel. Some gel systems are as clear as water, and others are turbid because the ingredients may not be completely molecularly dispersed (soluble or insoluble), or they may form aggregates, which disperse light.

- Tridax procumbens is a species of flowering plant in the daisy (Asteraceae) family. It is best known as a widespread weed and pest plant. The plant is native of tropical America and naturalized in tropical Africa, Asia, Australia. It is a wild herb distributed throughout India. It is annual or biennial somewhat patently hispid herbs. Stem branched, creeping at base, sub erect or trailing above. Leaves ovate-lanceolate, or elliptic- rhomboid, with a cineaste base, obtuse or sub-acute, coarsely serrate, or lobed, 2.5-7 cm long. Heads solitary, 1.2-1.5 cm across, on erect, 10-30 cm long peduncle. Marginal flowers 5-6 with pale yellow, 0.3 cm long ligules; disc flowers bright yellow. Tridax procumbens is found to possess pharmacological activities like hepatoprotective effect, immunomodulating property, promising wound healing activity, antidiabetic, hypertensive effect, antimicrobial, insect repellent activity, anti-inflammatory and antioxidant, bronchial catarrh, dysentery, diarrhea also prevent

falling of hairs and leads to hair growth promotion. From earlier research, it was already showed the presence of dexamethasone, luteolin, glucotureolin, β-sitosterol, flavone, glycoside, and quercetin in this plant. The plant has been established for the treatment of wound healing, dysentery, epilepsy, malaria, stomachache, diarrhea, high blood pressure, diabetes, hemorrhage, and metabolic syndrome. It also possesses insecticidal, anti-septic, parasiticidal and hepato-protective properties and has marked depressant action on respiration. It is a well-known ayurvedic medicine for liver disorders or hepato-protective nature besides gastritis and heartburn. This plant is also used as bio absorbent for removal of harmful Cr (VI) from the industrial wastewater.

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

- **Synonyms :-**

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

- **Taxonomic Classification :-**

Kingdom :- Plantae
Sub-kingdom:- Tracheobionta
Division :- Spermatophyta
Sub-division :- Magnoliophyta

Class :- Magnoliophyta
Subclass :- Asteridae
Order :- Asterales
Family :- Asteraceae
Genus :- Tridax
Species :- Procumben

- **Botanical Morphology** :-

1. **Habit :-**

Tridax procumbens is an annual or perennial herbaceous weed found in tropical and subtropical areas of the world, growing mainly during the rainy season at meadows, croplands, disturbed areas, lawns, roadside or settled areas. This medicinal herb shows a typical feature of a beneficial weed.

2. **Growth :-**

Plants are prostrate or erect forming patches, with flowering axis 15 to 35 cm high.

3. Leaves :-

Leaves are opposite, simple, carried by a petiole, 1 to 2 cm long. They are thick, soft and dark green. The lamina is oval to lanceolate, 2 to 6 cm long and 2 to 4 cm wide, base attenuate in the corner and with strongly and irregularly serrated margin Both sides are hispid, with tuberculate based bristles.

4. Inflorescence :-

Inflorescences in solitary capitulum held by a peduncle, 12 to 32 cm long, abundantly hispid. The bracts of the involucre are arranged in 2 rows. They are oval to lanceolate; 6 mm long, pubescent and green. Flower: Capitulum formed of 3 to 8 ligulate daisy like female flowers, creamy white on the periphery of capitulum, tridentate. In the center of capitulum, flowers are yellow, tubulate bisexual. The tube, 6 mm long, with five short tines at the top. This plant has two types of the flower as ray florets and disc florets with basal placentation.

5. **Fruit :-**

The fruit is a conical achene, 3.5 mm high, pubescent and brown to black at maturity. It is surrounded by a pappus of feathery bristles, horizontally prostrate at maturity.

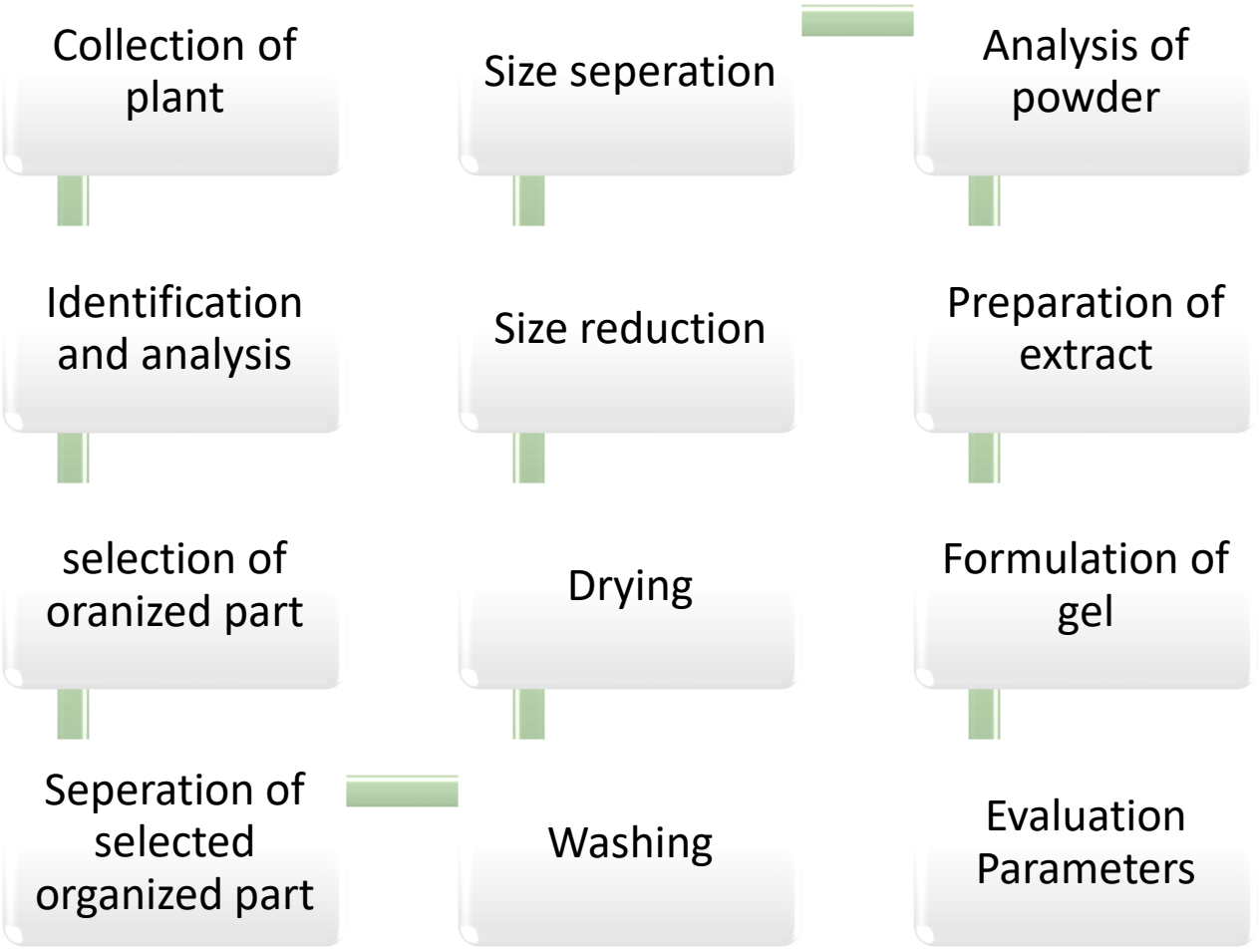
6. Stem and Root :-

Stem is cylindrical, hispid, covered with multi-cellular hairs of 1 mm; tuberculation at the base (Figure 7).The root is a strong taproot (Figure 8) system [26].

7. Genetics :-

The chromosome number of TRIDAX procumben L. has been registered as 2n = 36.

PLAN OF WORK :-



Experimentation :-

I. **Collection of plant** :-
Plant species collected from the Darodi village and surrounding hills nearby. The collected sample species were sent further for identification and analysis to confirm the collected species obtain is not adulterated and to meet our criteria.

II. **Identification and Analysis** :-
Now for the identification and confirmation of species the collected plant from its top flower part to root kept for drying to make herbarium. The herbarium of plant made on sheet of size 41cm*29cm. The herbarium of collected species sent for identification and analysis at BOTANICAL SURVEY OF INDIA (BSI), PUNE.

After collection of reports the obtained reports and certificate confirm the given herbarium species was found to be as Tridax procumben Linn. the reports are attached along with it.

III. Selection of organized \unorganized part :-
For experimental work and formulation the organized part of plant i.e. Leaves are selected.

IV. Separation of leaves :-

The leaves are directly handpicked from plant using gloves.

V. Washing :-

The leaves first washed with tapped water in order to remove the dirt settled on leaves. Now washed with distilled water 2-3 times to remove remaining impurity and kept them in vessel in order to drained the water from upper and lower surfaces of leaves.

VI. Drying :-

After draining of leaves now place the leaves on clean and dry sheet to dry. Kept the sheet under sunrays and dry the leaves. Regularly change the position of leaves and replace the sheet. About 5-7days require for complete evaporation and removal of water content.

VII. Size reduction :-

The size reduction of formed dried leaves done using mechanical mixer.

VIII. Size separation :-

The reduced size powder poured into sieve no.16 and fine powder obtained in the collector

IX. **Analysis of powder :-**

Now the obtained uniform sized powder its analysis done via process of loss of drying i.e. percentage moisture content and percentage ash value.

a. Ash value :-

Weigh accurately 2gm of powder sample and pour into already weigh crucible. Incinerate the material in a muffle furnace for 2 hrs. at 1000 degree celsius until free from all carbonaceous materials and ash is white or grayish white. Cool the crucible in a desiccator and weigh

% TOTAL ASH = {weight of ash in gram \ weight of sample taken in gram } * 100

b. Loss on drying (LOD):-

Take crucible and wash it thoroughly using distilled water and dry it in hot air oven. Weigh this clear and dry crucible and note as (W1). Weigh 2gm of powder and pour it into crucible and note the weight as (W2)

Place the crucible in hot air oven at 100 degree celsius. Now mark initial reading at 0 minute as it will be same as W2. At each time interval period i.e., at 15, 30, 45, 60,75 minutes...….and so on until and unless the consecutive readings of sample are same or minute difference of 0.1mg. Mark them as W3, W4, W5……up to the last weight noted and use the formula to calculate it.

% moisture content = {weight loss in gram \ weight of sample taken in gram} * 100

c. Acid insoluble ash value :-

The obtained ash from total ash boil for 5-10 minute with 25ml dilute hydrochloric acid. Filter and collect the insoluble matter on ashless filter paper. Wash the filter paper with hot water, ignite it tared crucible, cool and keep it in desiccator. Weigh the residue and calculate acid insoluble ash using formula.

% Acid insoluble ash = {Acid insoluble ash weight \ weight of sample} * 100

X. Extraction :-

The extract prepared using alcohol and distilled water in the proportion 1:10 and 1:20 of both the solvents.. The comparative study shows that's plant having higher rate of yield in distilled water Hence aqueous extract prepared using distilled water via maceration process. The macerated powder extracted collected and filter by using Whatman's filter paper. Collect the filtrate. Transfer filtrate into china dish and heat it on heating mantle collect the concentrated aqueous extract. Dry the extract and collect the pure extract of Tridax procumben L. and use this extract for formulation.

XI. Formulation :-

- **Procedure :-**

1. Required quantity of Carbopol was taken(as per batch wise formulation F1 & F2) and 10ml of water was added in it; it was stirred at 300-400 RPM in a homogenizer for 10 minutes.
2. After achieving a consistency (without any clumps of Carbopol) then add triethanolamine and more 10ml of water. Again, its stirred at higher 450 to 500RPM.
3. After another 10 minutes a gel base was formed then Tridax procumbens extract were added; and it was further stirred for 5 minutes.
4. PEG and Methyl Paraben were further added in accordingly in proportions to yield a homogenous gel.
5. Add the aloe vera to formulation and mix it thoroughly by again stirring. After all this process add peppermint oil to provide specific characteristic odour.
6. Finally this whole mixture was stirred for another 15 to 20 minutes with small increment of water and gel was formulated

❖ **Formulation table :-**

Sr.No.	Ingredients	Batch A (F1)	Batch B (F2)
1	**Tridax procumben extract**	**400mg**	**400mg**
2	**Carbopol 71G**	**1.2gm**	**1.2gm**
3	**HPMC**	**1.2gm**	**0.5mg**
4	**Triethanolamine**	**1ml**	**1.6ml**
5	**PEG**	**3ml**	**4ml**
6	**Methyl parabean**	**40mg**	**40mg**
7	**Aloe vera**	**5ml**	**4ml**
8	**Peppermint oil**	**0.4ml**	**0.4ml**
9	**Water**	**40ml**	**40ml**

XII. Evaluation of formulated gel :-

a) Anti-microbial study :-
b) Spreadibility
c) Ph
d) Viscosity
e) Appearance
f) Color and odour

A. **APPEARNCE :-**
Translucent (White)

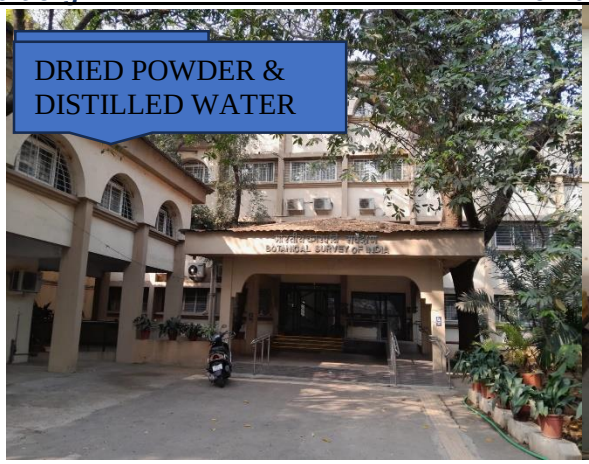
B. **COLOR & ODOUR** :_
Pale green (slight greenish white) with characteristics odour (peppermint)

C. **Ph :-**
The digital Ph meter used to detect Ph of formulated gels. The Ph found to be in range of 5.15 to 5.60.

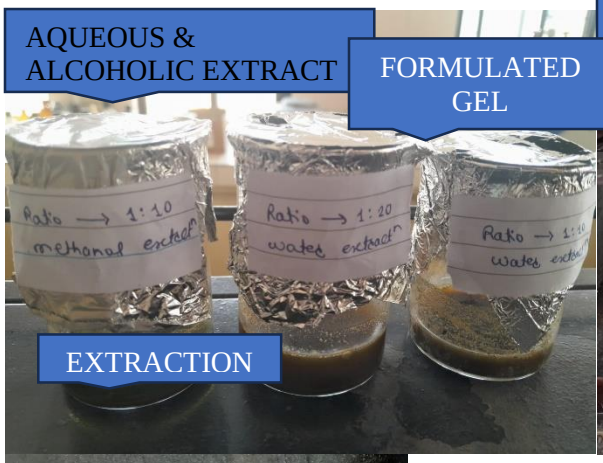
D. **VISCOSITY :-**
The gel viscosity measured by Brookfield viscometer and was found to be 1.4-1.8 cps.

E. **SPREADIBILITY :-**
The spreadibility check by placing gel in-between two glass plates and applying shear stress to determine the change in aur length covered by gel after applying the stress. The spreadibility found abour 5-6mm (good spreadibility)

F. **Antimicrobial activity :-**
The sample of Tridax extract and the formulated gel send for analysis of anti- bacterial activity fr the bacteria of gram (+) & gram(-) &pseudomonas. The obtained reports are attached along with it and results shows that significant anti-bacterial activity.

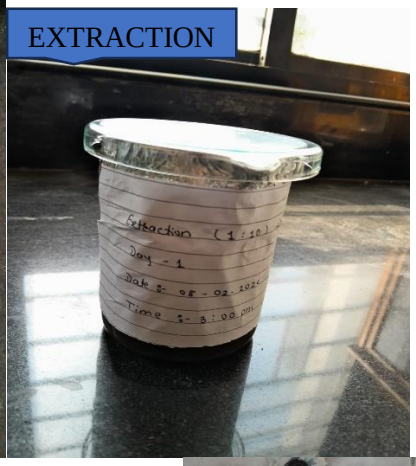

DRIED POWDER &
DISTILLED WATER


HEATING
MANTLE
MUFFLE FURNACE
Digital Bala


AQUEOUS &
ALCOHOLIC EXTRACT
FORMULATED
GEL
EXTRACTION

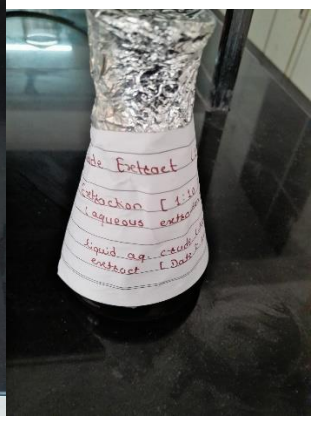

HOT AIR OVEN
COLLECTED
EXTRACT


ASH VALUE


EXTRACTION

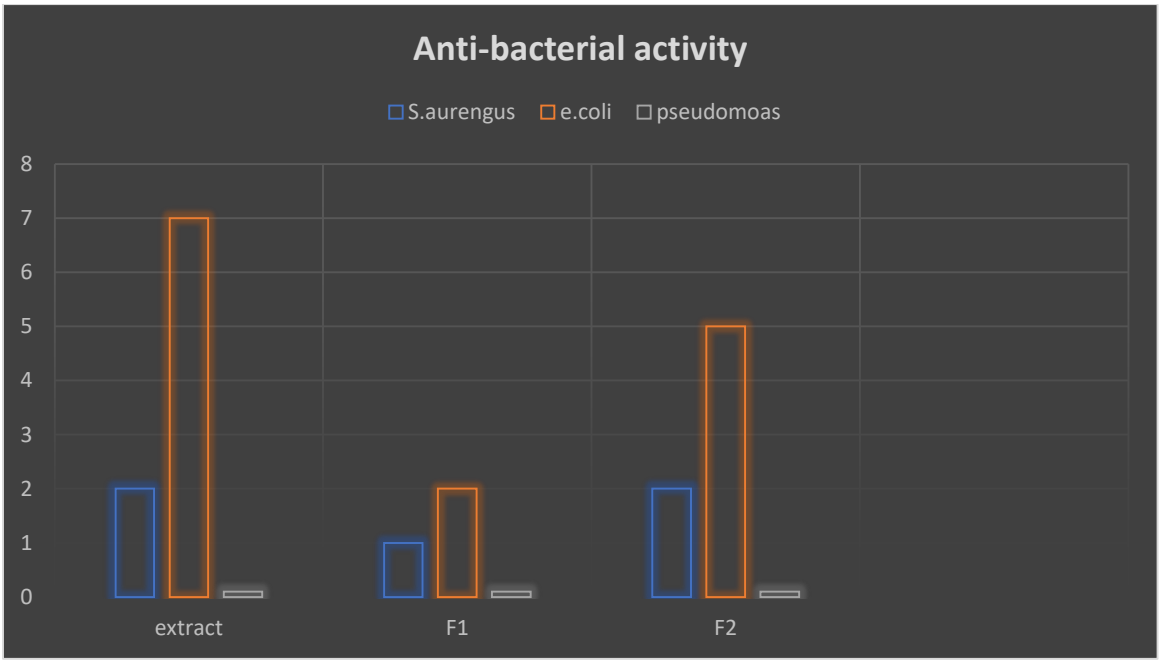

400
300
200
100
J-SIL
100 ml


LABLINE




Weighing balance
Homogenizer
Digital Ph meter
Dry powder formulation in mortar and pestle
Dried leaves
Evaporation of extract

Result :-



Sr.No.	Parameters	Outcome
1	% Ash Value	10 %
2	% Moisture Content	12.5 %
3	Acid insoluble ash %	3 %
4	Ph	5.15 to 5.90
5	Appearance	Transparent (white)
6	Viscosity	6-7mm
7	Color	Pale green (greenish white)
8	Odour	Characteristic (peppermint)

भारत सरकार/GOVERNMENT OF INDIA
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पश्चिमी क्षेत्रीय केंद्र/WESTERN REGIONAL CENTRE
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No.BSI/WRC/IDEN.CER./2024/07 Date: 08.02.2024

CERTIFICATE

This is to certify that **Mr. Pawade Ashish Uttam,** B. Pharmacy student from Allana College of Pharmacy, Pune, Maharashtra, the specimen/ specimens submitted by aforesaid is/ are identified by our expert/s as:

Specimen No.	Plant Name	Family
PAU 01	Tridax procumbens L.	Asteraceae

Pawar

(Madhuri Pawar)
Botanist
BSI, WRC, Pune

Advanced Scientific Research Laboratory
Department of Chemistry
Abeda Inamdar Senior College – Pune-411001

Date: 2/4//2024

Antimicrobial Activity Assay

(Well diffusion Qualitative Analysis)

REPORT

Generated for :-Ashish Uttam Pawade
Scientist Name: Mr. Kounsar Sheikh, Mr. Pranav Tambe**,** Ms. Neha Pai
Sample Detail: Tridax Extract and gel F1 and F2.

Methods

Materials: Incubator at 37˚C (Klenzone 2019), Pipettes of various sizes, sterile tips, 100 – 1000 µl, Multichanel pipette (Benchtop), Vortex mixer, sterile Petri dishes, Sterile flasks 100 – 1000 ml, Sterile 5ml disposable pippets, Sterile normal saline, Sterile Muller Hinton Broth-Cation adjusted (Himedia 2019),Cork borer sterile cotton swabs.

Medium: Sterile Muller Hinton Agar was used in this assay.

Use of standardized bacteria: Active cultures of bacterium Staphylococcus aureus (**ATCC 25932**) and Escherichia.coli(**ATCC8739**) Pseudomonas aeruginosa were used in this experiment.

Preparation of bacterial culture: Using aseptic techniques an isolated colony of bacteria was transferred into a 250 ml flask containing 100ml Muller-Hinton broth (2.1g of MH Himedia dissolved in 100ml distilled water digested and autoclaved at 15 psi for 20min), which was placed in the incubator overnight at 37˚ C. Broth suspension of bacterial cultures of Staphylococcus aurues and Escherichia.coli were and Pseudomonas aeruginosa ready after 24hrs of incubation. The optical density of the culture broth was adjusted to 0.5 McFarland Standard and recorded at 500 nm and the range obtained was between 0.5MFU (Cell density 1×10^8 CFU/mL) UV- Spectrophotometer (BioEra -2017). The final number of bacterial cells present on plate was adjusted to 1×10^5 CFU/mL.

Preparation of test compound and Dilutions: All the test compounds were crude extracts dissolved in DMSO as inert solvent.

Preparation of the plates: Plates were prepared under aseptic conditions. Sterile Muller Hinton agar was poured in sterile plates, 20ml media per plate and was allowed to solidify. 0.1ml of bacterial suspension was spreaded on each after cooling and solidifying of plates. well was made in each plate with the help of sterile cork borer and to each well 100ul of sample was loaded. Plates were kept for pre-diffusion at 4degree then incubated at 37 degree for 24 hours.Zone of clearance was observed after 24 hrs incubation. (Protocol was followed as recommended by CLSI protocol).

Results:

Zone of inhibition for Escherichia.coli(ATCC8739)

Compound/Drugs/Antimicrobial agent	Zone of inhibition
Tridax Extract	zone of inhibition seen(excellent)
F1	zone of inhibition seen(Mild)
F2	zone of inhibition seen(moderate)

Zone of Inhibition for Staphylococcus aureus (ATCC 25932)

Compound/Drugs/Antimicrobial agent	Zone of Inhibition
Tridax Extract	zone of inhibition seen(Excellent)
F1	zone of inhibition seen(mild)
F2	zone of inhibition seen(Moderate)

Zone of inhibition on Pseudomonas aeruginosa

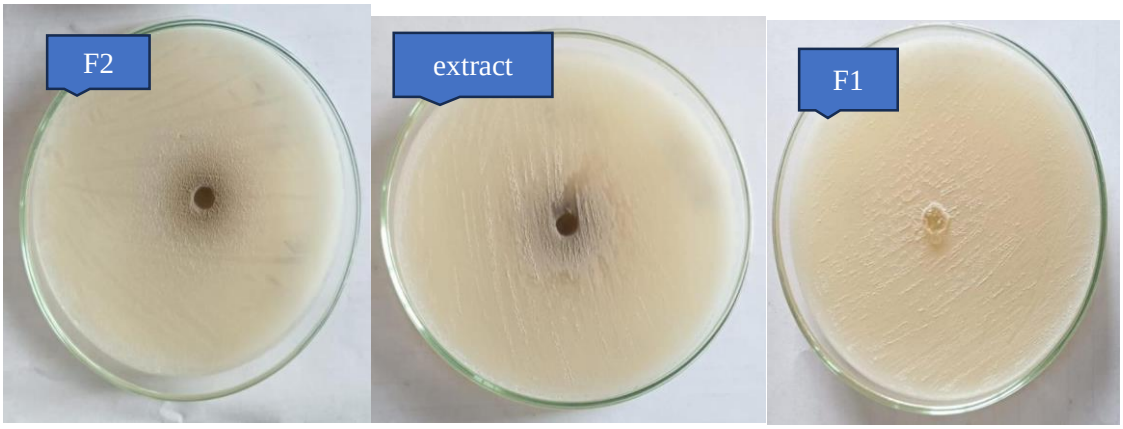
Compound/Drugs/Antimicrobial agent	Zone of inhibition
Extract	No zone of inhibition seen
F1	No zone of inhibition seen
F2	No zone of inhibition seen

Images of zone of Inhibition:

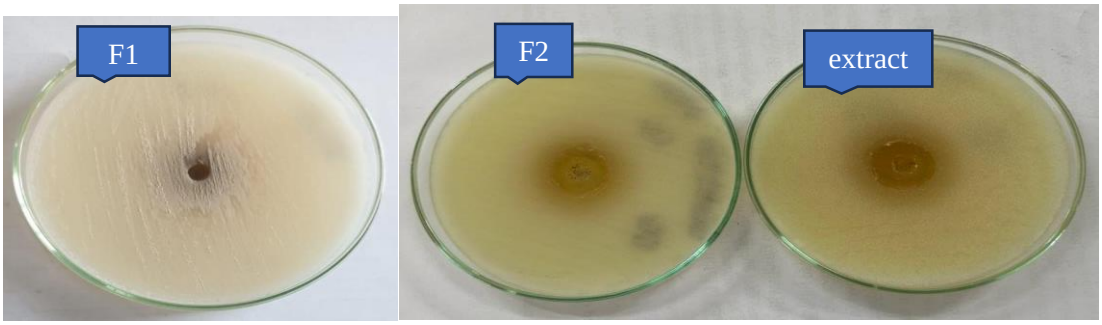
1. **Zone of inhibition on** Pseudomonas aeruginosa images

No zone of inhibition seen in all batch F1, F2 & extract

2. Zone of Inhibition for Staphylococcus aureus (ATCC 25932)



3. Zone of inhibition for Escherichia.coli(ATCC8739)



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