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IN-VITRO ANTIBACTERIAL EVALUATION OF *TRIDAX PROCUMBENS* EXTRACTS AGAINST DIFFERENT BACTERIAL STRAINS

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ABSTRACT

Bacteria are omnipresent, mainly free-living creatures that typically consist of a single biological cell (synonym: bacterium). Common bacterial diseases Pneumonia, Urinary tractinfections (UTI), Meningitis Wound infection, Cholera, Dysentery, Flesh eating disease, Tetanus, Anthrax, Leprosy, Plague, Typhoid & Tuberculosis (TB). Antibiotics kills or inhibitsthe growth or reproduction of bacteria. Antibacterial qualities can be found in heat, chemicalslike chlorine, and antibiotic medications. Nowadays, a lot of antimicrobial cleaning andhandwashing products are available. As an illustration, the unrefined extracts of curry, ginger,sage, mustard, garlic, cinnamon, and basil. *Tridax procumbens Linn* is a member of the*Compositae* family. There have been reports of a variety of pharmacological actions from *Tridax procumbens* extracts, including leishmanicidal, immunomodulatory, hepatoprotective,wound healing, and antiprotozoal characteristics. For this investigation, *Tridax procumbens* extracts in Ethanol and Aqueous were utilized. *Tridax procumbens Linn (L.)* leaves and rootextracts in Ethanolic and Aqueous were tested for their ability to inhibit the growth of*Escherichia coli, Salmonella typhi, Bacillus cereus,* and *Staphylococcus aureus.* Using the agarwell diffusion method, minimum inhibitory concentration by ELISA plate method and totalviability count. Antibacterial activity was examined by these methods. When compared to other organisms, the *Tridax procumbens* ethanol extracts elicit more potent suppression against*Salmonella typhi* and *Bacillus cereus*. Thus, it is possible to view *Tridax procumbens* leavesand root as a viable source of antibacterial chemicals.

KEY WORDS: Antibacterial action, Aqueous and Ethanolic extract, *Tridax procumbens L.*

INTRODUCTION.

Tridax procumbens L. (Asteraceae) has long been used as a traditional drink to treat liver disorders, diarrhoea, dysentery, and bronchial catarrh. This is despite reports that the plant mayinfect numerous crops. Hyderabad, Andhra Pradesh is home to *Tridax procumbens* stalks. Theplant produces daisy-like white or yellow blooms with three-toothed ray florets that have a yellow core. The leaves typically have an arrowhead form and are toothed. Scales or pappus are used to symbolize Calyx [1].

Fig:1 Tridax procumbens plant

SYNONYMS

• *Amellus pedunculata Ortega Ex wild,*
• *Balbisia divaricate Cass, Balbisia elongate wild,*

VERNACULAR NAMES

English	*Coat button/Tridax daisy*
Tamil	*Theta poodu*
Telugu	*Gaddi chemanthi*
Malayalam	*Chervenak*
Latin	*Tridax procumbens Linn*

USE OF TRIDAX PROCUMBENS

The uses of Wound healing, Bronchial catarrh, Antibacterial, Antimicrobial, Anticancer, Antiparasitic,Antifungal,Anti-inflammatory[2]

CHEMICAL COMPOSITION:

The plant is rich in sodium, potassium, and calcium. Leaves contain crude proteins = 26%;crude fibre =17%; and soluble carbohydrates = 39%, calcium oxide = 5% [3]

TAXONOMICAL CLASSIFICATION OF *TRIDAX PROCUMBENS* [4]

Kingdom	**Plantae**
Sub kingdom	**Tracheobionta**
Division	**Spermatophyte**
Sub-division	**Magnoliophyta**
Class	**Magnoliopsida**
Sub-class	**Asteridae**
Order	**Asterales**
Family	**Araceastee**
Genus	**Tridax**
Species	**Procumbene**

Table No1: Taxonomical classification of *Tridax procumbens*

PHYTOCHEMICAL CONSTITUENTS

Alkaloids, Terpenoids, flavonoids, Proteins and Cardiac glycosides are the phytochemicalconstituents present in the *Tridax procumbens* plant. [5]

PLANT PARTS

Fig 2: LEAVES

Fig3: FLOWER

Fig4: STEM

Fig5: ROOT

LEAVES

The leaves are annual and erect. The leaves are opposite, pointy, arrow-shaped, coarsely, anderratically toothed, yellowish-greenish, and hairy on the margins and stems [6]. Along with two typical compound leaf shapes (Pinnate and Palmate), six common shapes for basic leaves (generally Linear, Lanceolate, Oblong, Rhomboid, Elliptic, displayed.

FLOWER

Tridax procumbens flowers have white rays and yellow disc flowers. They are held on stemsabout 1-1.5 cm wide and 10-30 cm long. Flowering occurs in spring. [7]

STEM

Tridax procumbens is a perennial herbaceous plant with stems growing up to 20-75 cm long.The leaves of Tridax procumbens are opposite, pinnate, oblong to ovate and 2.5-5 cm long [8].

ROOT

Roots are strong rhizomes. The stem is cylindrical, strong, very capricious, covered with multicellular hairs 1 mm long, with a knot at the base. The leaves are opposite, simple, arrangedin petioles and 1 to 2 cm long. It is thick, soft and dark green [9].

MATERIALS AND METHODSCOLLECTION OF PLANTS

The leaves and root of the plant were collected in local areas of Coimbatore district, shade driedfor two to three days, and the herbarium were stored in the Department of Pharmacology of Sree Abirami College of Pharmacy.

AUTHENTICATION OF PLANT

The *Tridax procumbens L* plant authentication approved by Botanical Survey of India in Coimbatore (706).

EXTRACTION OF PLANT

Extracted using cold maceration method and applying invitro methods.

Aqueous extract

Take 25 gm of dried leaves and root, add 250 ml of distilled water, and kept for 48 hours. thenfiltration is done and boiled in the water bath for evaporation then the extract of plant is obtained [10].

Ethanolic extract

Take 25 gm of dried leaves and root, add 250 ml of Ethanol, and kept foe 48 hours. then filtration is done and boiled in the water bath for evaporation then the extract of plant is obtained [11].

FORMULA

PERCENTANGE YIELD **(%)** = Weight of the crude extract ×100

Weight of the sample

PHYTOCHEMICAL ANALYSIS

The phytochemical test shows the results of Proteins, Amino acids, Phenols, Alkaloids, Saponins, Tannins, Terpenoids, Flavonoids and Cardiac glycosides are present in plant part ofleaves and roots.[12]

ANALYSIS OF PHENOL COMPOUND

Take 1ml of extract, add 0.2ml of feline phenol reagent and 1ml of 20% sodium carbonates was added and incubate at 45°C for 45 mins. Then OD at 765nm. Using Microprocessor UV- Visible Spectrophotometer (single beam) [13].

NUTRIENT AGAR

Take 1.68gm of nutrient agar and 0.3 gm of agar-agar then dissolve with 60ml of distilledwater. Take the above solution in each plate.[14]

STERILIZATION

The media containing agar should be heated to dissolve the agar before autoclaving. The agar -agar media is kept in the autoclave sterilization for 15 mins in 121° C at 15 Lbs pressure.[15]

COLLECTION OF MICROBES

Bacterial strains such as *Escherichia coli, Salmonella typhi, Bacillus cereus, Staphylococcus aureus* was used for the study and were collected from CBNR laboratory Coimbatore. The collected microbes were maintained in nutrient agar broth and cultured in nutrient agar medium[16].

SELECTION OF MICROBES:

The gram-positive bacteria -*Bacillus cereus, Staphylococcus aureus*

The gram-negative bacteria- *Escherichia coli, Salmonella typhi*

ANTIBACTERIAL ACTIVITY BY AGAR WELL DIFFUSION METHOD

The agar well diffusion method was used to study the antimicrobial activity of the sample *Escherichia coli, Salmonella typhi, Bacillus cereus, Staphylococcus aureus* of 80µl were takenand spread on the nutrient agar plates, followed 3 wells were with cork borer and samples wereadded (Aqueous extract, Ethanolic extract and DMSO) to the respective well along with positive control. (Antibiotic Disc-Methicillin) [17].

INCUBATION OF MICROBES:

After the media preparation of the media then it is allowed to be cooked in the autoclave. To evaluate the antibacterial activity of the samples, the diameters of the inhibition zone were measured and noted. [18]

MINIMAL INHIBITORY CONCENTRATION AND VIABLE COUNT ASSAY

This method was initiated by preparing nutrient broth using sterile 96-well plates (Wiegand etal. 2008). The wells of each row were filled with 0.1 ml sterilized nutrient broth. Sequentially, sample in different concentration of 10,20,30,40,50µl along with 10µl of the culture *B. cereus*,and *S. typhi* respectively. The plate was incubated for 24h at 37°C. The resulting turbidity wasobserved, after 24h MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 600nm with a 96 well ELISA plate reader(Robonik). % of cell death was calculated using the formula. [19]

Percentage (%) of cell death = Control OD-Sample OD X 100

Control OD

After the calculation the higher percentage of cell death occurred, concentration was selected to identify the viability. Nutrient agar was prepared, and the treated sample (10µl of low concentration, High concentration, and control cells) was added to the centre of the sterilepetri plate and poured nutrient agar and mixed. plate was incubated for 24 to 48hours and the number of the bacteria was counted to finalize the optimum concentration to resist the bacterialgrowth [20].

RESULTS & DISCUSSIONEXTRACT VALUE

S.NO	WEIGHT OF SAMPLE (gm)	SOLVENT	IMMERSION TIME	WEIGHT OF CRUDE EXTRACT (gm)	PERCENTAGE YEILD (%)
1	25	AQUEOUS	5 DAYS	1.3	5.2%
2	25	ETHANOL	5 DAYS	1.6	6.4%

Table:2 Extraction value

The table (2) shows the result of extracted *Tridax procumbens* leaves and roots by coldmaceration method.

PERCENTAGE YIELD:

Percentage yield aqueous**: 5.2%**

Percentage yield ethanol:**6.4% [18]**

PHYTOCHEMIAL TEST

S.NO	PLANT CONSTITUENTS	TESTS	AQUEOUS	ETHANOL
1.	Proteins	Biuret test	+	-
2.	Amino acids	Ninhydrin test	+	-
3.	Tannins	Ferric chloridetest	+	+
4.	Phenols	Lead acetate test	+	+
5.	Alkaloids	Mayers test	+	+
6.	Saponins	Forth test	+	+
7.	Terpenoids	Salkowski test	+	+
8.	Flavonoids	Alkaline reagent test	+	+
9.	Cardiac glycosides	Keller killiani test	+	+

Table:3 Phytochemical test results

TOTAL PHENOL TEST

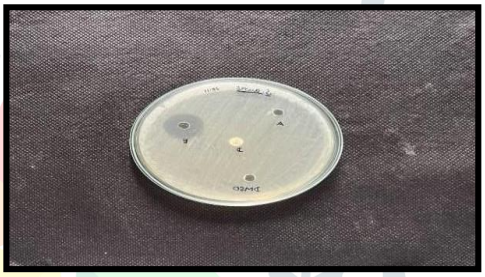
The phenol test is for conforming the Antioxidant and Antibacterial activity. The opticaldensity of different concentration is shown below in the table (4).

S. No	Samples	OD Readings	CONCENTRATION (µg/ml)
1.	Blank	0	0SS
2.	Aqueous	0.402	40
3.	Ethanol	0.642	62
4.	Standard (gallic acid)	0.998	90

Table:4 Total phenol content test resultANTIBACTERIAL SENSITIVITY TEST(ABST)

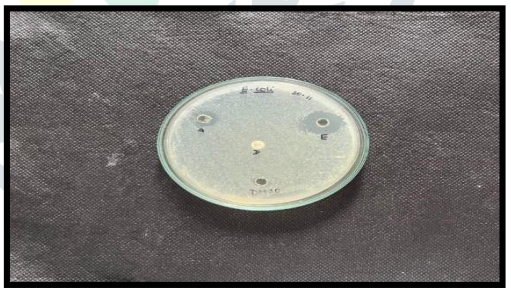
The ABST of different strains of bacteria are to be shown below in the fig: 6. *Bacillus cereus,salmonella typhi, Staphylococcus aureus* and *Escherichia coli.*

Bacillus cereus



Staphylococcus aureus

Salmonella typhi



Escherichia coli

Fig6: ANTIBACTERIAL SENSITIVITY TEST(ABST)

The zone of inhibition is shown in the table (5). *Bacillus cereus* and *Salmonella typhi* has themaximum zone of inhibition in the ethanolic extract.

S. No	Name of the organism	Zone of inhibition (in mm)			
		Aqueous	Ethanol	Disc	DMSO
1.	*Escherichia coli*	12	18	0	0
2.	*Staphylococcus aureus*	0	18	0	0
3.	*Bacillus cereus*	22	26	0	0
4.	*Salmonella typhi*	0	26	0	0

Table:5 Zone of inhibition for ABST

The graphical representation of aqueous and ethanolic extract antibacterial activity against *E. coli*, *S. aureus*, *B. cereus* and *S. typhi* are shown below in the table (6).

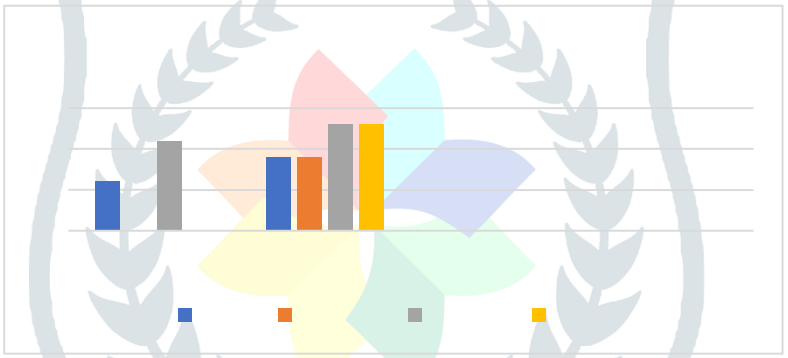


Table:6 Graphical representation of ABST

Comparing all the bacterial strains *Salmonella typhi* has the superior zone of inhibition.Which shows the antibacterial potential of the *Tridax procumbens*.

MINIMAL INHIBITORY CONCENTRATION

BACTERIA NAME	BLANK	CONTROL	10µl	20µl	30µl	40µl	50µl
B. cereus	0	0.677	40.91%	42.68%	46.82%	47.41%	57.31%
S. Typhi	0	0.630	18.48%	24.60%	34.12%	40.00%	42.20%

Table:7Minimum Inhibitory Concentration value

The superior activities shown by the *B. cereus* and *S. typhi* is Minimum InhibitoryConcentration is represented in the table (8).

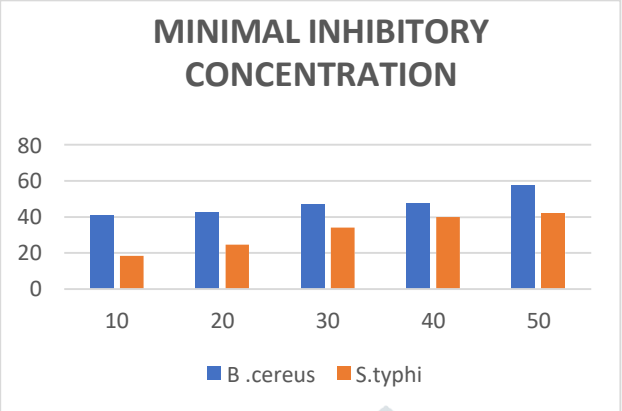


Table:8 Graphical representation of MICTOTAL VIABLE COUNT

The concentration of *salmonella typhi* shows the bacterial growth. The sample treated with *salmonella typhi* the inhibition of growth of bacteria. This indicates the presence of antibacterial property [21].

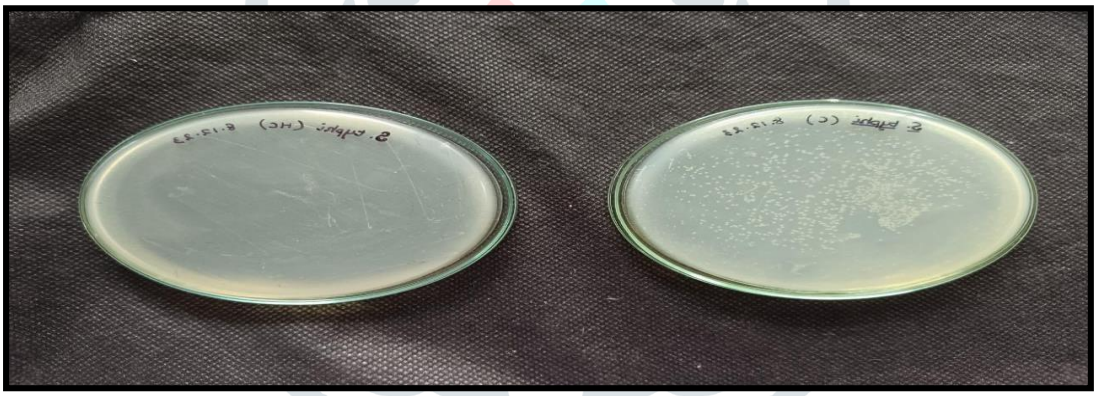


Fig18: *salmonella typhi*

The concentration of Bacillus cereus shows the bacterial growth. The sample treated with Bacillus cereus the inhibition of growth of bacteria. This indicates the presence of antibacterialproperty [22].

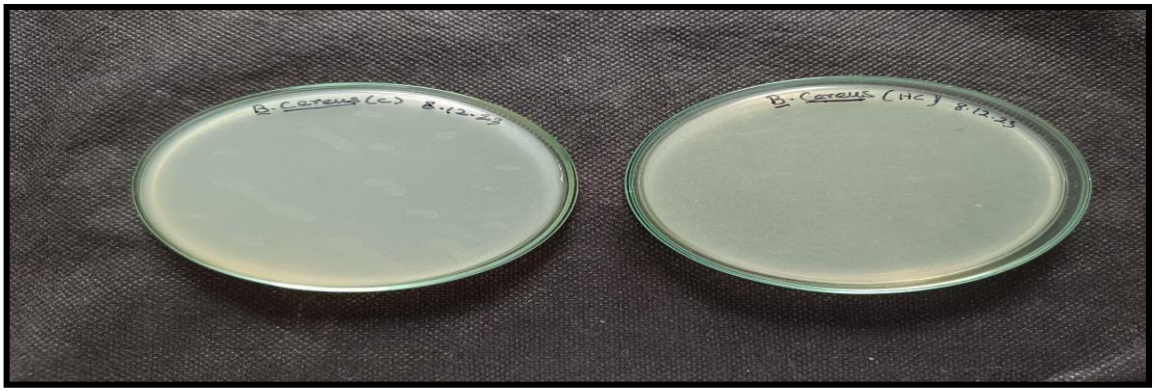


Fig 19: Total viable count (*B. cereus*)

S. NO	SAMPLES	CONCENTRATION	TREAT
1.	*Salmonella typhi*	2 Numerus	NIL
2.	*Bacillus cereus*	20	NIL

Table:10 Total Viable Count value

SUMMARY & CONCLUSION

Based on the literature survey, we have selected *Tridax procumbens* plant leaves and roots forour study and planned to analyse the Antibacterial effect by ABST, MIC (ELISA) and TVC. The extraction of the plant is done. The percentage yield is for aqueous is 5.2% and ethanol is6.4%. Phytochemical analysis of aqueous extract shows the presence of protein, amino acid, tannins, flavonoids, saponins, terpenoids, phenols and cardiac glycosides. Ethanolic extract shows the presence of tannins, phenols, alkaloids, terpenoids, flavonoids, cardiac glycosides and saponins [23].

Test for phenol is to be positive and determine the optical density by UV-Spectrophotometer. The ABST test for zone of inhibition is superior in *Bacillus cereus* and *salmonella typhi*. *Salmonella typhi* and *Bacillus cereus* are taken for the ELISA test and resultsof minimal inhibitory concentration analysed. The minimal inhibitory concentration of both the bacteria is taken to determine the total viability count [24].

Salmonella typhi - control was numerous growths of bacteria and treated was shows theinhibition of bacteria. *Bacillus cereus* - control was (20) number of bacteria growth and treated was shows the inhibition of bacterial growth. The investigation of antibacterial activity of *Tridax procumbens* was proved to be positive by antibacterial sensitivity test thezone of inhibition represents the plant has the property of antibiotic [25].

Then the Elisa test the formation of zone is superior in *Bacillus cereus* and *Salmonella typhi*

so minimal inhibitory concentration was done with these two strains which shows the total

viability count that *Salmonella typhi* and *Bacillus cereus* in the control the bacterial growth wasformed then in the sample treated bacterial media the bacterial viability was not shown this testgives the best results of inhibition. Several studies already conducted in leaves part of this plant.In our study shows better results obtained by using root part of plant along with leaves. Even though further *in-vivo* studies needed for this plant in future [25].

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