



**PHARMACOGNOSTICAL EVALUATION, ANTIMICROBIAL AND ANTIOXIDANT  
POTENTIAL OF *TRIDAX PROCUMBENS* L.**

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**ABSTRACT**

Modern medicine is facing the worst ever existential crisis in the form of antibiotic/antifungal resistance. The wide spectrum antibiotic resistance observed is now posing a serious public health concern, with medical scholars warning of a return to the pre-biotic era. Progressive increase in antimicrobial resistance has given rise to the need to investigate other sources of therapy such as medicinal plants. *Tridax procumbens*, the weed is a source plant which is investigated for its *Krimighna* and *Rasayana* guna, the properties being mentioned in various Ayurvedic texts and here it is tried to prove it on scientific parameters. Plant extracts were screened for their antimicrobial activities using the Disc diffusion method. Their phytochemical contents were screened, FRP and TAC methods were used to assess their antioxidant activities. HPTLC reveals the presence of Beta sitosterol in plant sample. Three plant extracts, viz. aqueous, alcoholic and hydroalcoholic(50%) of *Tridax procumbens* L. were used to assess antimicrobial activity against potentially pathogenic three bacterial (*P. aeruginosa*, *S. aureus*, *E. coli*) and 3 fungal strains (*C. albicans*, *M. furfur*, *T. rubrum*). Inhibitory activity of the extract was seen against *T. rubrum*, *M. furfur* and *P. aeruginosa* but remarkable activity was seen in case of *C. albicans* showing 11mm zone of inhibition against 15 mm ZOI by standard(Ketoconazole) disc. The water and hydroalcoholic extracts showed zone of inhibition against *C. albicans* only. Phytochemicals like alkaloids, phenols, flavonoids, saponins, tannins, protein and carbohydrates were found in plant extracts, and the plant extract also demonstrated remarkable antioxidant activity.

**KEYWORDS:** *Tridax procumbens*, *Bhringraja*, *Krimighna*, *Rasayana*, *C.albicans*, *M. furfur*, *T. rubrum*, *P. aeruginosa*, *S. aureus*, *E. coli*, Beta sitosterol.

**INTRODUCTION**

Ayurveda which is regarded as the *Upaveda* or subscription of *Atharva veda*, is the collection of valuable knowledge enscripted by Indian Sages thousands of years ago stating the fundamentals of Ayurveda. It recognized the use of medicines for diseases far before anyone else in the world and even today, the basis of most modern medicine still boils down to the wisdom of Indian medicinal practices of years gone by. If we dig deep in the history, India, ancient home of cosmic wisdom, has actually always had a leading reputation in the field of medicine. One of the basic fundamental of Ayurveda treatment as stated by *Acharya Charaka* in his *Samhita*<sup>[1]</sup> –

प्रयोजन चास्य स्वस्थस्य स्वास्थ्यरक्षणमातुरस्य विकारप्रशमनं च ॥

Which means curing the diseased and restoring the health of otherwise healthy person, but in today's busy life people, in hurry of getting rid of diseases are

unaware about the ill effects of unnecessary drug and doses they are taking which ultimately is deteriorating their health again. Irrational use of drugs in treatment has make the society prone to drug resistance and ultimately more diseased.

In this long run Antibiotic resistance has emerged as a burning global problem due to irrational use of medicine in an attempt to cope with bacterial and fungal diseases. It is the ugliest outcome of advance antibiotic era. Moreover the use of Broad spectrum antibiotics have worsen the situation. While everyone today is trying to find solution of this problem, let us realize that answer in question is already existent in the Ayurvedic texts written by Ayurvedic scholars thousands of years ago. While, outwardly it might seem unconvincing that how a book of such ancient times can actually answer a problem of today, but, deep study would suggest that every word in the texts were written after much thinking and understanding. The intense analysis that went behind

the creation of these texts has given immortality to the words in them. The microbes which we know today after the invention of microscopes only, was a known fact and has been mentioned by our *Acharyas* as *Bhoot/Jantu*<sup>[2]</sup> in their *Samhitas* and not only this, they have even successfully treated them with their applied knowledge. Due to various reasons, known and unknown, the modern world was blinded or ignorant to our Ayurveda texts as well as precious herbs mentioned in them but at present everyone is trying to find the solution through Ayurveda. In western world now, the use of herbal medicines is steadily growing with approximately 40 per cent of population reporting use of herb to treat medical illnesses within the past year. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine.<sup>[3]</sup> So we can rely on our ancient texts for solving the present crisis and for that we aimed to work on antimicrobial properties of *Tridax*

*procumbens* which is being assumed as wild variety of *Eclipta alba* (*Bhringraja*) in our Ayurvedic texts and has been praised for its antimicrobial activity.

## MATERIAL METHOD

### Material

The plant materials taken for the study was:

- Genuine sample of whole plant of *Tridax procumbens* Linn.
- The genuine samples of whole plant of *Tridax procumbens*(Linn) were collected from Herbal garden, Rishikul campus, Haridwar.

### Methods

#### 1) Herbarium<sup>[4]</sup> and authentication of collected genuine plant material

Collected dried, pressed plant was mounted on herbarium sheet bearing detailed data label and stored in a herbarium cabinet in a climate controlled fumigated closed room.

#### Details of Collection of Genuine Samples and authentication of *Tridax procumbens*(Linn).

S.N.	Name of the Plant	Time of Collection	Place of Collection	Authentication of Herbarium
1.	<i>Tridax procumbens</i> (Linn)	July 2017	Haridwar, Uttarakhand	NBRI, Lucknow (LWG-102997)

## 2) Pharmacognostic Study

### i) Macroscopic study

All the collected genuine samples were dried and studied macroscopically with naked eye, magnifying lens and measuring tape with the help of Pharmacognostical parameters i.e shape, size, surface, colour, odour and taste and findings were recorded.

### ii) Microscopic study<sup>[5]</sup>

Transverse section of plant specimen of whole plant of *Tridax procumbens* (Linn) viz. Root, Stem and Leaf was investigated for establishing the identity of drug and Powder microscopy done to evaluate different constituents by using different staining.

## METHODOLOGY

Specimens were soaked in water or other solvents depending upon the hardness of the sample and transverse sections were taken using sharp razor blades. Numerous temporary and permanent mounts of the microscopic sections of the specimen were made and examined microscopically. Different staining reagents were applied on transverse sections so as to differentiate between different cell wall components.

Similarly Powder Microscopy with whole plant sample of *Tridax procumbens* Linn. was examined while staining with different chemical reagents using Methylene blue, Saffranin, and glycerine and then by observing under electron microscope.

**3. Preliminary phytochemical screening<sup>[5]</sup>:** Methods as per API followed.

## 4) Qualitative Phytochemical Screening<sup>[6]</sup>

### 4.1-Determination of total phenol content

A stock solution (1mg/ml) of the methanol extract is taken in 25 ml volumetric flask and 10 ml distilled water and 1.5 ml folin's reagent were added. After keeping the mixture for 5 min. 4 ml of 20% Na<sub>2</sub>CO<sub>3</sub> is added. The volume is made upto 25 ml with distilled water. Mixture kept for 5 min. and absorbance is taken at 765 nm. Standard curve was prepared using Gallic acid (0.1 mg/ml) in the same way of stock solution. All determinations were carried out in triplicate (table no. 3). The total phenolics components in the extracts in gallic acid equivalents (GAE) were calculated by the following formula:  $T = C \times V/M$ ; where T = total phenolic contents, milligram per gram of 22 sample extract, in GAE; C = the concentration of gallic acid established from the calibration curve, mg/mL; V = the volume of extract, milliliter; M = the weight of sample extract (g).

### 4.2-Determination of total flavonoid content

From the similar stock solution 0.5 ml of the extract was taken into the test tube and 0.5 ml of 2% AlCl<sub>3</sub> added and the volume was made upto 5 ml with methanol. Absorbance taken at 420 nm. Standard curve was prepared using Quercetin (0.1 mg/ml) in the same way of stock solution. All determinations were carried out in triplicate. The total flavonoid components in the extracts in quercetin equivalents (QCE) were calculated by the following formula:  $T = C \times V/M$ ; where T = total flavonoid contents, milligram per gram of sample extract, in QCE; C = the concentration of gallic acid established from the calibration curve, mg/mL; V = the

volume of extract, milliliter; M = the weight of sample extract (g).

## 5) Method of antimicrobial screening<sup>[7]</sup>

### Instrument/material used for antimicrobial study

Nose mask, Hand gloves, Screw cap test tube, Weighing machine, Inoculating loop, Petri dishes, Pipettes, 50 ml and 100 ml bottles, Inoculating loop, Filter paper discs, Sterile forceps, Sterile cotton swabs, Autoclave, Laminar flow with Bunsen burner, Dimethyl sulphoxide (DMSO), Nutrient broth, Potato dextrose agar, Incubator, Streptomycin, Ketoconazole, Aqueous, Alcoholic and Hydroalcoholic plant/sample extracts.

### Steps involve in antimicrobial study includes

**5.1. Extract preparation:** For extraction, the method described by Ndip *et al.* (2007) was employed and three different extracts were prepared namely

- a) Aqueous extract
- b) Ethanolic extract
- c) Hydroalcoholic extract (50%)

### 5.2. Preparation of fresh bacterial culture

Fresh bacterial cultures were prepared by sub-culturing stock bacterial cultures into freshly prepared nutrient agar and incubating at 37°C for 24 hours. These 24-hour-old bacterial cultures were transferred into freshly prepared nutrient broth and standardised to 0.5 McFarland turbidity standards using the spectrophotometer to obtain the desired cell density of 1.5 X 10<sup>8</sup> (cells/ml).

### Principle of disc diffusion method

The agar diffusion assay is one method for quantifying the ability of antibiotics to inhibit bacterial/fungal growth. The agar diffusion test, or the Kirby-Bauer disk-diffusion method, is a means of measuring the effect of an antimicrobial agent against bacteria/fungi grown in culture. The bacteria/fungi in question is swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to effective concentration. This is the zone of inhibition, this along with the rate of antibiotic diffusion are used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection (Mohanty A *et al.*, 2010).

### 5.2.1 Test Organisms

#### 5.2.1.1 Gram Negative Bacteria

- *Pseudomonas aeruginosa*
- *E.coli*

#### 5.2.1.2 Gram Positive Bacteria

- *Staphylococcus aureus*

#### 5.2.2.3 Fungi

- *Candia albicans*
- *Malessazia furfur*
- *Trichophyton rubrum*

### 5.3. The Culture Medium and Its Composition

Nutrient agar was used to conduct the antimicrobial screening using the disc diffusion method. The nutrient agar PDA was bought from the market Nutrient agar contains the following substances:

#### 5.3.1 Ingredients (Gm/L)

Dextrose- 20 g

Potato Extract - 4 g\*

Agar- 15 g

Final pH (at 25°C) 5.6±0.2

\*4.0gm of potato extract is equivalent to 200gm of potato infusion

#### 5.3.2 Preparation of the Medium

First of all, the amount of nutrient agar needed was calculated and then added to distilled water in an agar bottle and mixed thoroughly. It was then autoclaved to dissolve the agar and sterilize it.

### 6. Preparation of Test Plate

1. The test organisms were transferred from the subculture to petri dish containing the required amount of melted and sterilized agar medium as required by the size of the dish.
2. The bacterial and fungal suspension was taken by a loop and mixed with normal saline with the help of vortex machine.
3. Then a sterilized cotton bud was taken and dipped into the bacterial/ fungal suspension. Then the bacterial/fungal sample is applied to the petri dish with the help of this cotton bud.
4. The swabbing was done carefully so that the microorganisms would be spread out evenly on the dish.

### Type of Discs

#### 6.1 Standard Discs

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation Streptomycin and Ketoconazole (30µg/disc) standard disc was used as the reference.

#### 6.2 Blank Discs

These were used as negative controls, which ensure that the residual solvents (left over the discs even after air-

drying) and the filter paper were not active themselves. Here the negative control used was methanol.

### 6.3 Sample Discs

1. In a specific volume of solvent, measured amount of each test sample was dissolved to obtain the desired concentrations in an aseptic condition (5, 10 and 20 mg conc. of extract used)
2. For the each extract of husk, a stock solution of 10mg/ml was prepared and was used directly.
3. Sterilized filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of 10µl of test samples and dried.

### 6.4 Diffusion and Incubation

Here, incubation is done for maintaining controlled environmental conditions for the purpose of favouring growth or development of microbial or tissue cultures or to maintain optimal conditions for a chemical or immunologic reaction.

1. The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi.
2. The plates were then inverted and kept in an incubator at 37°C for 24 hours.

### 7. Determination of Antimicrobial Activity by Measuring Zone of Inhibition

In Agar diffusion test, the size of the zone of inhibition indicates the degree of sensitivity of bacteria to a drug or testing agent. In general, a bigger area of bacteria-free media surrounding an antibiotic disk means the bacteria are more sensitive to the drug or the testing agent the disk contains. The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs,

## OBSERVATION AND RESULTS

### A) Pharmacognostical Study

#### 1. Organoleptic Study

S.N.	PART	COLOUR	ODOUR	TASTE	SHAPE	TEXTURE
1.	ROOT	Light brown	-	-	Cylindrical	Hairy
2.	STEM	Greenish	-	-	Cylindrical	Rough
3.	LEAF	Greenish	characteristic	Bitter	Lanceolate to ovate	Thick and hairy

#### b) Organoleptic characters of powder of whole plant (W.P) of (*Tridax procumbens*)

Parts	Colour	Odour	Touch	Taste
W.P. of <i>Tridax procumbens</i>	Greenish yellow	Odourless	Coarse	<i>Katu</i>

### 2. Macroscopic Study

**Description-** *Tridax procumbens* is a decumbent perennial herb.

#### Taxonomical Classification<sup>[10]</sup>

The plant classification details are-

Kingdom:- Plantae- Plants

Sub-kingdom :- Tracheobionta- Seed Plants

Division:-Magnoliophyta- Flowering plants

Class:- Magnoliopsida- Dicotyledons

which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

### 8. Antioxidant Assay Determination<sup>[8]</sup>

**8.1-Total antioxidant capacity:** a stock solution of extract (1mg/ml) was prepared. Four different concentrations of the extracts (50, 100, 150 and 200 µl) were make up volume 3ml with working reagent solution (sulphuric acid, sodium phosphate and ammonium molybdate). The tubes containing the reaction solution were boiled for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid (0.1 mg/ml) with methanol.

**8.2-Ferric Reducing power assay<sup>[9]</sup>:** A stock solution of extract (1mg/ml) was prepared. Different concentrations of the extracts (50, 100, 150 and 200 µl) were make up volume 1ml with distilled water then mixed with 2.5 ml phosphate buffer solution and 2.5 ml of 1% potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] in test tubes. The mixture was incubated at 50° C, for 20 min. Then 2.5 mL of 10% trichloroacetic acid was added to the mixture and mixed thoroughly and then 0.5 mL of FeCl<sub>3</sub> (0.1% solution) was added and allowed to stand for 10 min. Then the absorbance of this mixture was measured at 700 nm using a UV-VIS spectrophotometer. The higher the absorbance of the reaction mixture, the greater the reducing power. Ascorbic acid (0.1 mg/ml) was used as a positive control.

Sub class:- Asteridae

Order:- Asterales

Family:- *Asteraceae*- Aster Family

Genus :- *Tridax* L-Tridax

Species:- *Tridax procumbens* (L) coat button.

**Derivation of specific name:** *Tridax* refers to the 3 lobes of the ray flowers, *procumbens*: called due to trailing habit or stems lying along the ground procumbent.<sup>[11]</sup>

Leaves are opposite, hairy, often deeply lobed; heads solitary, with very hairy involucre bracts bearing basal placentation.<sup>[12]</sup> Flowers are of 2 kind; ray flowers 5 or 6, female, with narrow corolla tube and brown ligulate limb, white or pale yellow. Disc flowers many, the corolla narrow campanulate, 8 mm long, bright yellow and hairy at the top, with spreading pappus of plumose hairs.<sup>[13]</sup> Flowering and fruiting throughout the year.

**Habitat:** Weeds of gardens, pastures, often in short grasslands.

**Distribution:** Throughout India. Annual to perennial herb with decumbent stem upto 0.5 m long that lie along the ground and curve upwards near the tip.

**Root:** Tap root, shallow and cylindrical.

**Stem:** Herbaceous, cylindrical, decumbent and branched with distinct nodes.

**Leaf:** Leaves are opposite, 3-7 cm long, 1-4 cm wide, lanceolate to ovate, with reticulate venation having characteristic odour and acrid taste. Appearance of leaf is roughened scabrous with irregularly toothed margin, acute apex, and wedge shaped base.

**Petiole:** Petiole is green, short and easily fractures.

**Inflorescence:** A terminal heterogamous head and receptacle of the head is convex and surrounded by green involucre.

**Flowers:** In solitary or at the forks of dichotomy; cream to yellow. Compound, daisy-like flowers are composed of yellow disk florets and 4 white ray florets. The tubular florets occupy the centre and the ligulate florets are found at the margin. Flowering throughout the year. The tip of the petal-like appendage of the ray florets is divided into 3-4 rounded lobes. Flowers attractive to butterflies and bees.

**Fruits:** Dry, indehiscent fruits are known as Achenes. The brown, oblong fruits (2 mm long) are covered in silky hairs and have a 5-6 mm long pappus (a feathery

appendage which helps the fruit to disperse by wind). Fruiting throughout the year.

**Seed:** With pendulous embryo, non endospermous.

### 3. Microscopic Study

#### (i) Microscopic characters of different parts of *Tridax procumbens* in transverse section (T.S)

**a. Root:** Transverse section through root is almost circular in outline. Outermost 8-10 layers of cork is followed by secondary cortex and secondary phloem. Phloem consist of patches of stone cells and phloem parenchyma. Cambium is distinct 5-6 layers, central portion occupied by secondary xylem. Xylem consist of group of broad and narrow lumen vessels. Medullary rays are distinct, broad and parenchymatous.

**b. Stem:** T.S. of stem is circular in outline. Outermost layer is single layered epidermis covered by cuticle with multicellular simple trichomes. Few trichomes are 'T' shape. Hypodermis 2-3 layered, sclerenchymatous followed by 6-8 layered collenchymatous cortex. Endodermis is distinct, single layered. Several collateral and open vascular bundles arranged in a ring. Each vascular bundle capped with fibrous bundle sheath. Phloem and xylem are well developed, interrupted by 3-4 layers of cambium. Schizogenous mucilage canals are present near vascular bundle, toward pith. Central portion is occupied by collenchymatous pith.

**c. Leaf:** T.S. leaf is dorsiventral showing protuberated mid rib and side veins. T.S. passing through mid rib region shows single layered thick walled upper and lower epidermal cells. Simple, multicellular trichomes are present on lower side of mid rib. Midrib shows meristele composed of well developed phloem and xylem. Remaining tissues are collenchymatous. T.S. passing through surrounding meristele region consist of tannin containing cells present in ring. T.S. passing through lamina region show single layered upper and lower epidermis. Cells of upper epidermis are much larger as compared to lower epidermis. Beneath them are present palisade cells which are columnar and single layered followed by 6-7 layered spongy mesophyll cells.

### 4. Powder Microscopy Study

#### Powder microscopy of whole plant of *Tridax procumbens* (Linn.)

Features	W.P of <i>Tridax procumbens</i> (Linn)
Starch	+
Calcium crystals	+
Cellulose	+
Mucilage	+
Cutin	+
Cell nuclei	-
Lignine	+

#### Powder analysis of *Tridax procumbens* (Linn)

It is dark green, fine, odourless powder with slight bitter taste. Powder microscopy reveals the presence of

multicellular covering trichome, anisocytic type of stomata, calcium prismatic crystal, xylem vessel and phloem fibres. Powder shows the spiral thickenings,

vascular bundles, elongated palisade cells and thin walled parenchyma cells.

## 5. Physicochemical Study

### Physicochemical study of whole plant of *Tridax procumbens*(Linn)

S. N.	PHYSICOCHEMICAL TEST	<i>Tridax procumbens</i> (Linn)
1.	MOISTURE CONTENT	12.45% w/w
2.	EXTRACTIVE VALUES	
	a. AQUEOUS EXTRACTIVE VALUE	15.5%
	b. ALCOHOL EXTRACTIVE VALUE	8.75%
3.	ASH VALUES	
	a. TOTAL ASH	13.85%
	b. WATER SOLUBLE ASH	3.25%
	c. ACID INSOLUBLE ASH	1.8%

## 6. Phytochemical Analysis of *Tridax Procumbens* (Linn)

### 6.1. Qualitative Phytochemical Analysis

S.N.	PHYTOCONSTITUENTS	<i>Tridax procumbens</i> (Linn)
1.	Alkaloids	+++
2.	Phenols	+++
3.	Flavonoids	++
4.	Saponins	++
5.	Carbohydrate	++
6.	Tannins	+
7.	Steroid	+
8.	Proteins	+
9.	Terpenoids	-

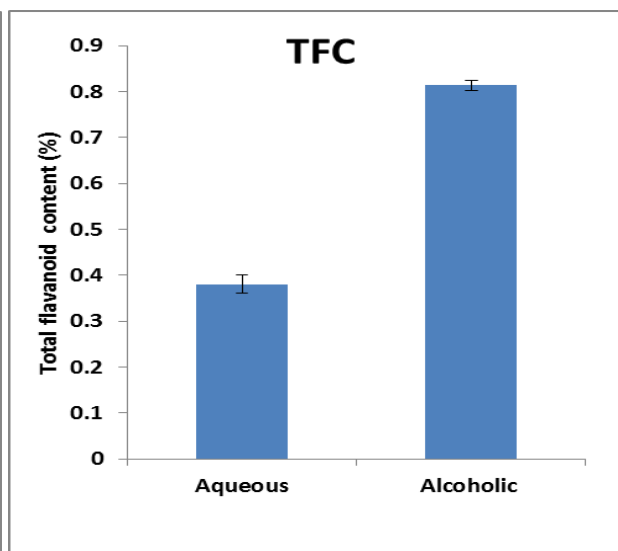
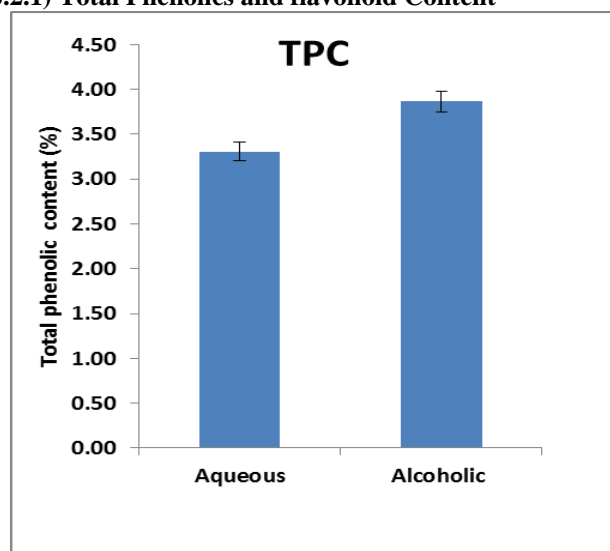
### 6.2 Quantitative Phytochemical Analysis

We selected two phytoconstituent for quantitative analysis as they were fairly present in qualitative analysis

and are further well known for their antimicrobial and antioxidant action and their mean % was calculated which is as follows-

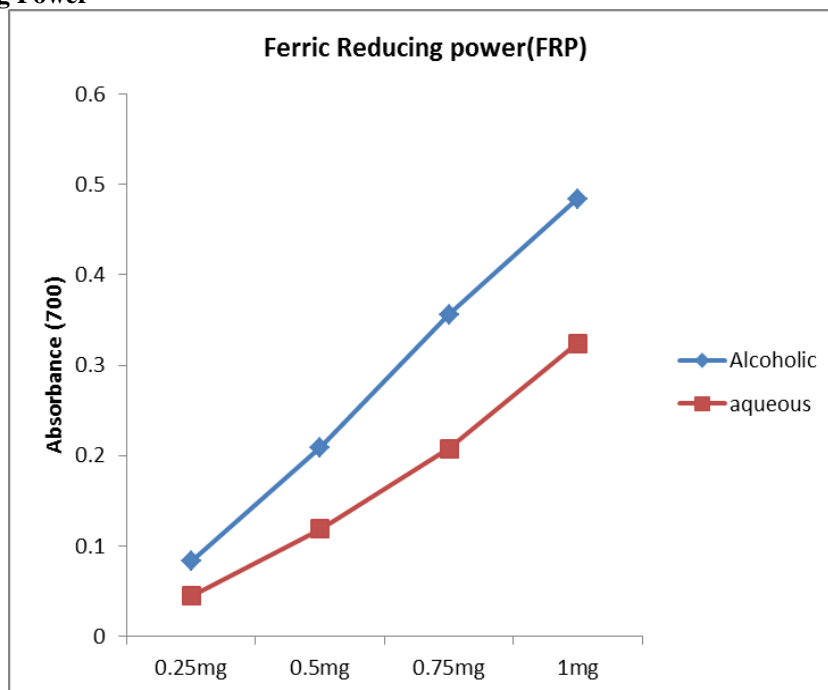
S.N.	PHYTOCONSTITUENTS	<i>T.procumbens</i> (L.) (Aqueous extract)	<i>T.procumbens</i> (L.) (Ethanol extract)
1.	Total phenolics	3.30 %	3.86%
2.	Total flavonoids	.38%	.81%

#### 6.2.1) Total Phenolics and flavonoid Content

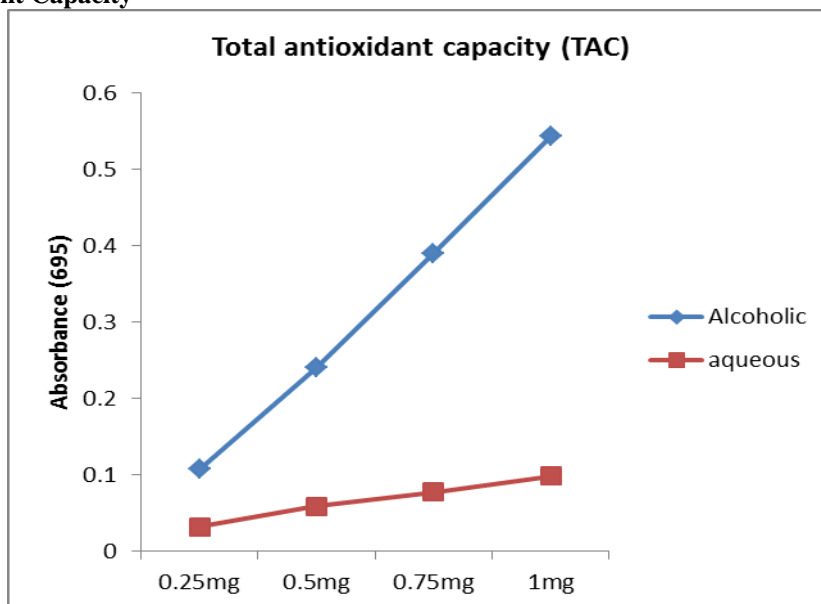


7. Antioxidant Assay

7.1 Ferric Reducing Power



7.2 Total Antioxidant Capacity



8. Chromatographic Study

8.1. TLC (Thin Layer Chromatography) analysis for different samples

Solvent system: Toluene: Methanol: Formic acid (7.8:2.2:0.75 ml)

Sample: Ethanol Extract

Visualization: Iodine Vapour

Retardation Factor ( $R_f$  Value) Calculation of *Tridax procumbens* (Linn) [Table no.6]

NAME OF SAMPLE (ETHANOL EXTRACT)	$R_f$ value
1 <sup>st</sup> spot(beta sitosterol)	.34
2 <sup>nd</sup> spot	.40

8.2 HPTLC

S.N.	SAMPLE NAME	STANDARD	QUANTITY
1.	<i>Tridax procumbens</i> (Linn)	Beta-sitosterol	46.24 µg

As per the findings, 46.24 µg Beta sitosterol is present in 10 mg sample so the estimated concentration of Beta sitosterol is **4.624 µg/mg** extract.

9. Antimicrobial Screening

Antimicrobial activity of different extracts of *Tridax procumbens* on different micro organisms.

S.N.	Fungal Strains	Ethanol Extract	Water Extract	Hydroalcoholic Extract	Control
1.	<i>Candida albicans</i>	++++	+	++	+++++
2.	<i>Malassezia furfur</i>	+	-	-	+++++
3.	<i>Trichophyton rubrum</i>	+	-	-	+++++
4.	<i>Pseudomonas aeruginosa</i>	+	-	-	++++
5.	<i>Staphylococcus aureus</i>	-	-	-	+++
6.	<i>Escherechia coli</i>	-	-	-	++++

Inhibition is recorded on diameter of growth inhibition zone: 0, d = < 5mm. (No Activity); +, d = 5 to 6mm; ++, d = 6 to 8mm; +++, d = 8 to 10 mm; +++++, d = 10mm to 15mm; ++++++, d = 15 to 20mm; Ketoconazole and Streptomycin disks were used as control for fungal and bacterial strains respectively.

Here maximum activity was seen against *Candida albicans*'s ethanolic extract with ZOI of 11mm, which is 15 mm for standard ketoconazole. ZOI measured for *M. furfur* and *T. rubrum* was 6mm and for *P. aeruginosa* it was 4mm.

Study Photographs

Macroscopic Characters of *Tridax Procumbens*



Figure 1: *Tridax procumbens* (Whole plant).



Figure 2: Flower of *Tridax procumbens*.



Herbarium Sheet for Authentication.



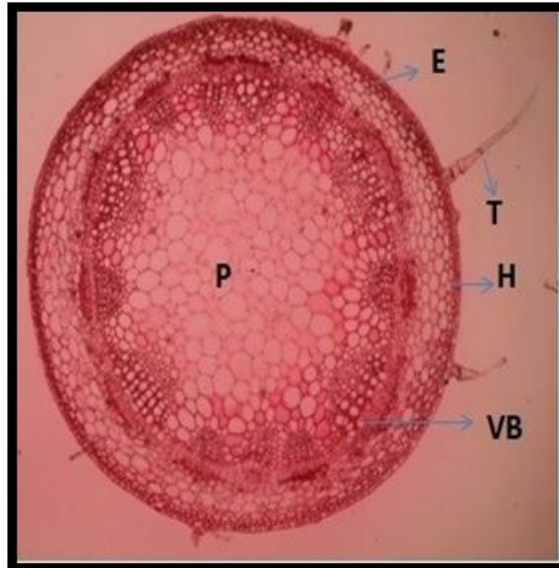
Herbarium Authentication Certificate(*T. Procumbens*).



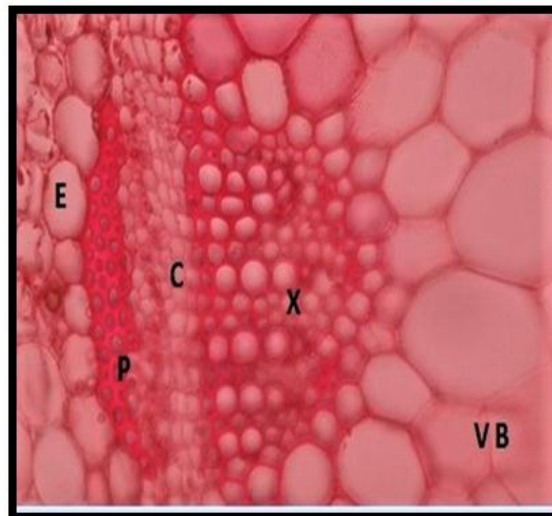
T.S. Root with Distinct Medullary Rays & Well Developed Cork.



Root Pith Region Occupied By Sec. Xylem with Scattered Stone Cells.



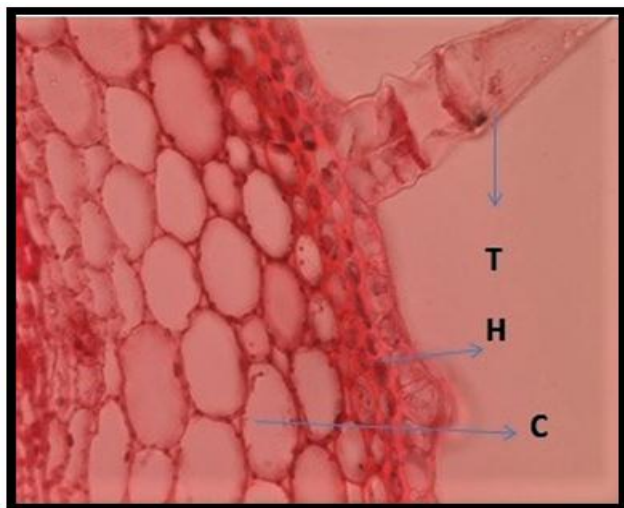
T.S. Stem: with Collateral & Open V.B. in Ring.



Single Vascular Bundle in Stem.



T.S. of Leaf with tannin cells.



Trichome of stem.

C-cork, SC-secondary cortex, P.- phloem, SX-secondary xylem, S-stone cell, Ttrichome, Epepiblema.



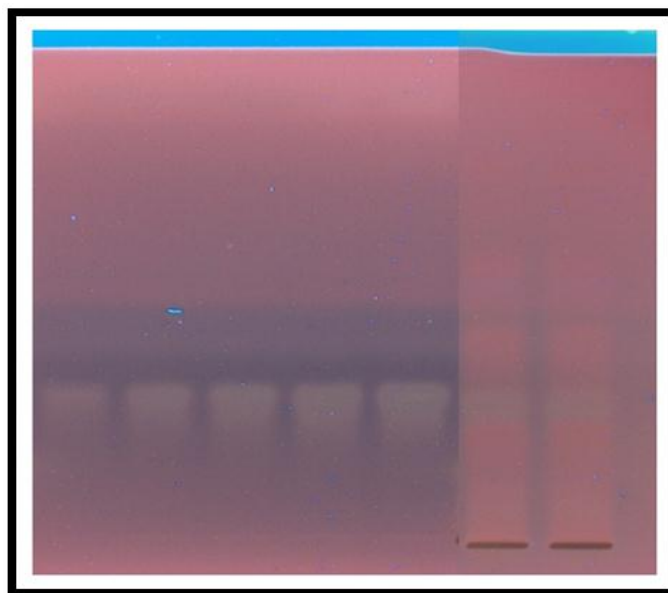
TLC at white light



TLC at 254 nm



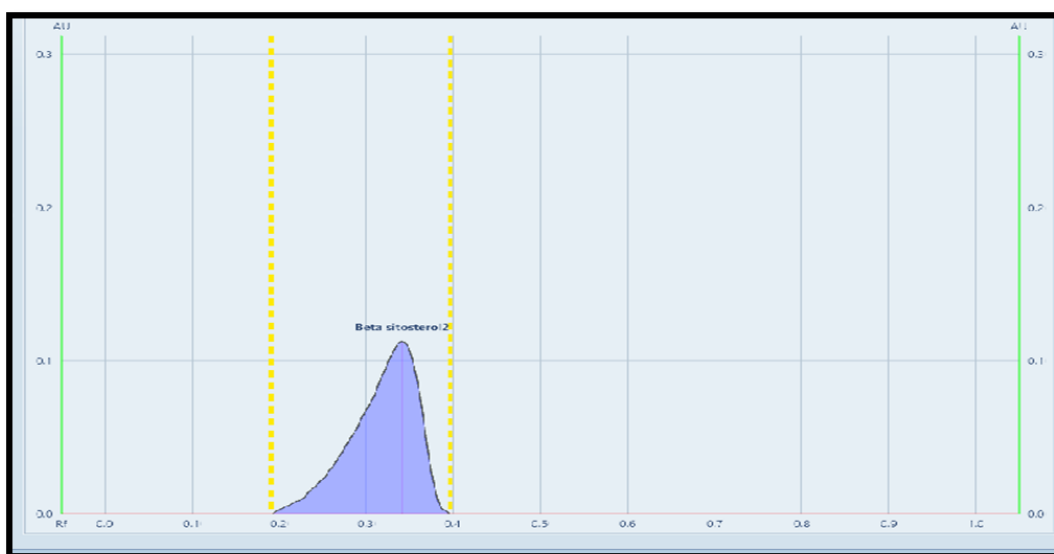
TLC at 366nm



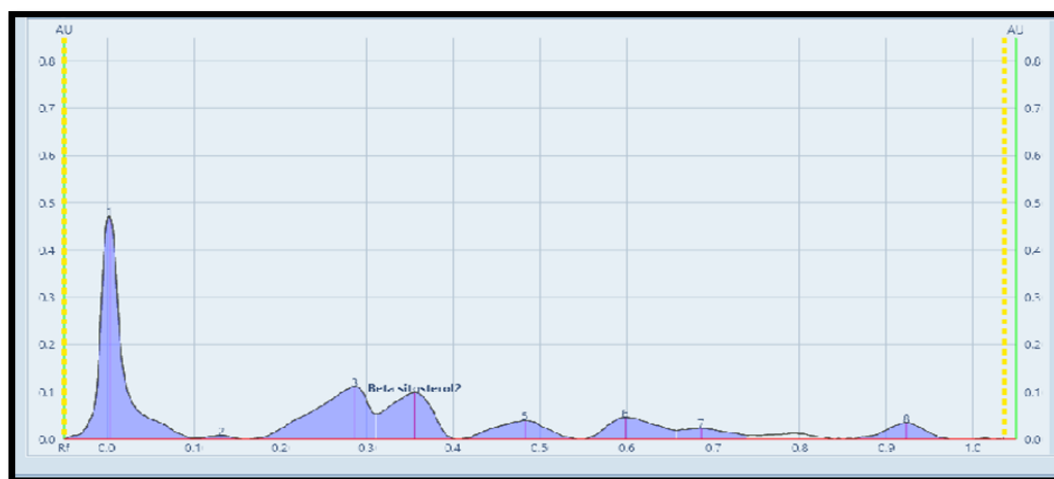
HPTLC Chromatograph at 366nm After Derivatization.



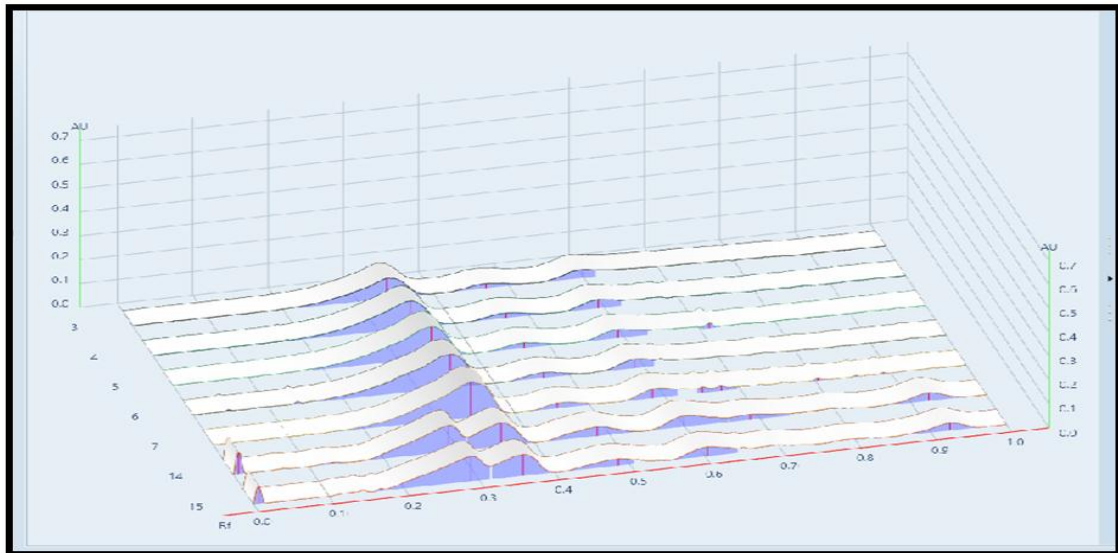
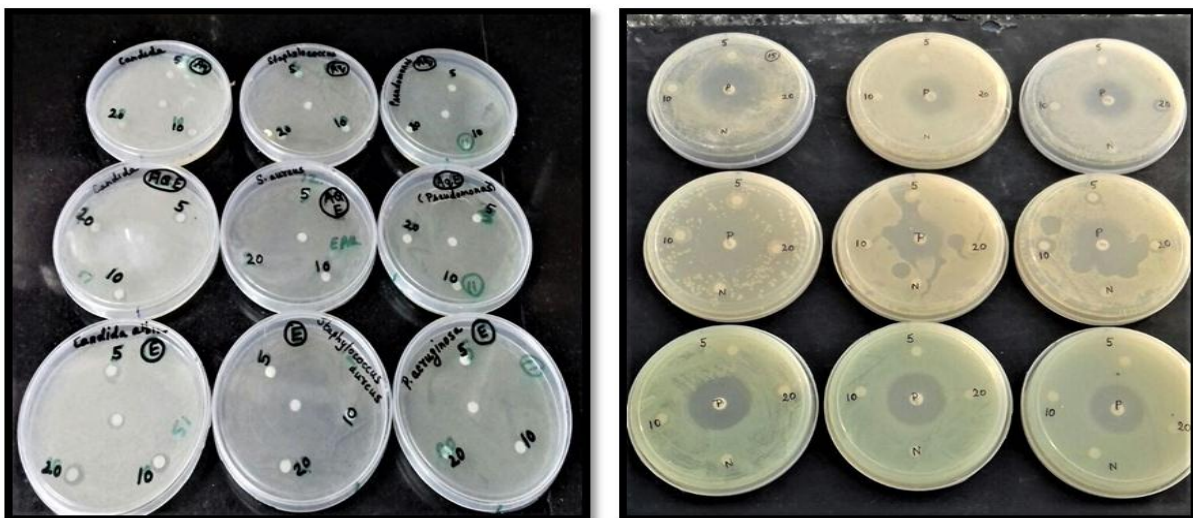
Hptlc Chromatograph in Visible Light After Derivatization.



Hptlc Finger Print of Beta-Sitosterol.



HPTLC Finger Print of *T. procumbens*.

3D Densitometric HPTLC Chromatogram of *T. Procumbens*.

Discs Showing Zone of Inhibition.

### CONCLUSION

The study was aimed to establish the antimicrobial and antioxidant activity of *Tridax procumbens*. This study investigated the antimicrobial, antioxidant and phytochemical content of the plant extract which demonstrates the presence of important phytochemicals like alkaloid and phenol in abundance along with a fair amount of flavonoid, carbohydrates and saponin and lesser tannin, steroid and protein. The plant extract, especially the ethanolic extract exhibited excellent absorbance with increasing concentration in FRP and TAC tests, thus proving it to be rich in antioxidant. In antimicrobial study, activity of three plant extracts visually aqueous, alcoholic and hydroalcoholic were seen against 3 bacterial (*P. aeruginosa*, *S. aureus*, *E. coli*) and 3 fungal strains (*C. albicans*, *M. furfur*, *T. rubrum*). The growth inhibiting zones were studied and reported that the chief dermatophytes namely *Candida albicans*, *Trichophyton rubrum*, *Malassezia furfur* and *Pseudomonas aeruginosa* were diminished by the

ethanolic plant extract. Though the activity against *T. rubrum*, *M. furfur* and *P. aeruginosa* was not significant but remarkable activity was seen in case of *C. albicans* showing 11mm zone of inhibition against 15 mm ZOI by standard (Ketoconazole) disc in Disc Diffusion method. The hydroalcohol extract showed little activity against *C. albicans* only and aqueous extracts shows minimal insignificant activity, and that was too against *Candida* only. No activity was seen against *E. Coli* and *Staphylococcus aureus* with any extract. The work experimentally proves and advocates the future use of plant extract in fungal disease caused by *C. albicans*. Thus inspite being a neglected weed *Tridax procumbens* affirms itself a novel gift of nature to mankind thus proving the golden words of Acharya Charaka<sup>[14]</sup>.

नानौपधिभूतं जगत्किञ्चिद् व्यमुपलभ्यते तां तां युक्तिमर्थं च तंतमभिप्रेत्य" ॥ (च.सू. 26/12)

ie. there is nothing on the earth which is not a medicine, and it only depends how we use it. Thus it is an ideal

medicine being safer, cost effective and easily available and the present study concludes that the extract of *Tridax procumbens* may be used in developing phytomedicines with antifungal properties along with serving a rich antioxidant source for future.

### Recommendations

- As we get better results with ethanolic extract of *Tridax procumbens* as compared to aqueous extract, we could hope for getting more fascinating results by extraction with more different solvents other than water like ether, hexane, chloroform etc.
- We did our study with whole plant which in future could be focussed on separate or specific plant parts, as plant metabolites could have been in different percentage in diff. plant parts.
- The disc diffusion method was not a conclusive method to determine the antimicrobial content of the plant extracts. A further conclusive test like broth microdilution methods and others to determine the MICs and bactericidal concentrations of the plant extracts needs to be tried.
- Activity on more different microbial strains can be tested.
- In vivo study and toxicity test needs to be done before use in humans.
- Further research needs to be carried out to isolate and identify the active compound(s) responsible for their antimicrobial and antioxidant properties.

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