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Comparison of Leaves and Stem Aqueous Extract of Tridax Procumbens for Antimicrobial Activity

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Abstract

In present study, aq. extracts of leaves and stem part of Tridax Procumbens were compared for antimicrobial activity. Firstly, all organoleptic and physicochemical properties of leaves and stem powder were evaluated and it shows that drug is pure and having required constituents sufficiently. Aq. extract of leaves powder and stem powder was prepared separately using Soxhlet extraction technique. Both the extracts were evaluated for physiochemical screening using standard procedures. Aq. extract of leaves shows presence of tannis, saponins, anthocyanin, coumarins, alkaloids, proteins, amino acids, diterpenes, phytosterol, cardial glycosides, phlobatannins and flavonoids. Aq. extract of stem shows presence of tannins, coumarins, phenol, cardial glycosides and flavonoids. Both extracts were primarily evaluated for antimicrobial activity against E.coli and S. aureus by selecting dose of 500 mg. Finally, antimicrobial assay was performed for both extracts (500mg) against E.coli and S. aureus by well diffusion method using Amoxicillin and Amikacin as standards respectively. Antimicrobial assay shows that aq. extract of stem part is more effective against S. aureus than E.coli; leaves aq. extract also shows antimicrobial activity against S .aureus and E.coli. From present studies we can conclude that aq. extract of stem and leaves of Tridax Procumbens having antimicrobial activity and stem extract is more effective against S. aureus as compared to leaf extract.

Keywords: Tridax procumbens, Soxhlet extraction, Antimicrobial, Amoxicillin, extracts

Introduction

India has rich history of traditional System of Medicine based upon six systems, out of which Ayurveda stands to be the most ancient, most widely accepted, practiced & flourished indigenous system of Medicine. The systems of Medicine in India are Unani, Siddha, Homeopathy, and Yoga & Naturopathy.[1] Ayurvedic research involved different steps like authentication (stage of collection, Parts of the plant collected, regional status, botanical identity like phyto-Morphology, Phyto-Morphology Microscopical & histological analysis tor taxonomical identity etc.), Foreign Matter determination (herbs collected should be free from soil, insect part of animal excreta etc.) and Organoleptic evaluation. The pharmacological treatment of disease began long ago the use of herbs.[2] Tridax Procumbens belongs to the family Asteraceae or compositae, T. balbisiodes and T. trilobata are the other species of the genus. It is an annual perennial weed from Central America and found throughout in India. Especially in Maharashtra, Madhya Pradesh and Chhattisgarh regions as weed. It is often rooting at nodes with solitary, long stalked, Yellow composite,

heterogamous, bisexual flower with white flowering heads & very harry, coarsely toothed, petiolate, ovate or lanceolate leaves. Whole aerial part is useful medicinally, leaves possess wound healing insecticidal, hypotensive action while seeds are used to Control bleeding.[3] Antimicrobial activity can be defined a collective term for all active principles (agents) that inhibit the growth of bacteria. Prevent the formation of microbial colonies and may destroy Microorganisms. [4] The Tridax Procumbens showed strong anti-microbial activity against Staphylococcus aureus (Gram-positive) and E.coli (Gram-negative). [5, 6]

Materials and Methods

Collection of plant material

Plant specimen was collected during February 2022; the leaves and stem of Tridax Procumbens L. were collected from local area Rase Chakan, Maharashtra. Then the fresh leaves and stem were washed with water to remove any soil, dust and other impurities. The whole plant is collected for preparation of herbarium.

Identification and Authentication

The plant specimen was dried by pressing it between the sheets of paper. After that herbarium sheet was prepared and was submitted Botanical Survey of India, Koregaon park, Pune for authentication. It was authenticated by Botanist D. L. Shirodkar with Specimen voucher no. BSI/WRC/Iden. Cer. /2022/0104220016824.

Drying of plant materials

After collection of plant materials, it was cleaned by removing unwanted parts away from leaves and stem. After separation these parts were washed with water and sundried for 2-3 days. After that it was stored in an air tight container away from moisture for further study.

Grinding of plant materials

The dried leaves and stems of Tridax Procumbens were then grinded into a fine powder by using Grinder. The powders of leaves and stem were then stored in different containers for further study.

Powder characteristic study of plant material

The crude drug powders were evaluated for parameters like color, odour, total ash value, acid insoluble ash value, water soluble ash value, loss on drying etc.[7, 8]

a) Determination of total ash value

A shing involves an oxidation of components of the product. A high ash value is indicating the combination, substitution, or adulteration.

Procedure

1. The silica crucible was weighted and tared.
2. About 2 g of the powdered drug weighed into the silica crucible.
3. Then heated with the help of burner using a flame about 2 cm high and crucible was supported about 7 cm above the flame. Heated till vapours cease to be evolved, then crucible was lowered and heated more strongly until all the carbon is burnt off.
4. Crucible was cooled at room temperature.
5. The ash was weighed and the percentage of total ash with reference to air dried sample of the crude drug was calculated.

b) Determination of acid insoluble ash value

Hydrochloric acid insoluble ash is the residue obtained after extracting the sulphated or total ash with hydrochloric acid. This acid insoluble ash value mainly indicates combination with siliceous materials like earth or sand.

Procedure

1. The ash from the crucible used for total ash value was washed with 25 ml of dilute Hydrochloric acid into 100 ml beaker and boiled for 5 min.
2. Then it is filtered through filter paper and residue was washed twice with hot water.
3. Crucible ignited in the flame, cooled and weighed.
4. Filter paper along with residue kept into the crucible and heated gently until vapours cease to be evolved and then strongly until all carbon is removed.
5. Cooled at room temperature.

6. Residue was weighed and acid insoluble ash of the crude drug with reference to the air-dried sample of the crude drug was calculated.

c) Determination of water-soluble ash value

Water soluble ash is the part of total ash content which is soluble in water. It indicates the previous exhaustion of crude drug or the incorrect preparation.

1. The ash obtained from total ash value was boiled with 25 ml of water in 100 ml beaker.
2. Then it is filtered through filter paper and residue was washed twice with hot water.
3. Crucible ignited in the flame, cooled and weighed.
4. Filter paper along with residue kept into the crucible and heated gently until vapours cease to be evolved and then strongly until all carbon is removed.
5. Cooled at room temperature.
6. The weight of incinerated residue was subtracted from the weight of total ash.
7. The difference of weight was considered as the water-soluble ash.
8. The percentage of water-soluble ash was calculated with reference to the air-dried sample of drug.

Loss on drying

1. 2 g of the powdered crude drug was weighed into a weighed flat and thin porcelain dish.
2. Dried in the oven at 1000 C.
3. Cooled at room temperature and weighed.
4. The loss in weight is recorded as moisture.

Extraction of plant material

The powdered stem and leaves used for the extraction purpose. The extraction of both the powders was carried out simultaneously by using hot continuous extraction process i.e., Soxhlet extraction. The 30 g of powder extracted with distilled water in Soxhlet extractor for 12 hr at temperature of 500C. Then the extracts were evaporated to dryness on electric water bath. Then the extract obtained was weighed and its percentage with reference to air dried sample of plant material was calculated. The collected extract was kept in tightly closed container for further study

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Figure No.1: Soxhlet Extraction Assembly

Preliminary investigation of prepared extracts[9]

The crude extracts were observed for parameters like percentage yield, color and nature.

Phytochemical screening of crude aqueous extracts

The extract of Tridax Procumbens stem and leaves were tested for the presence of phytochemicals such as tannins, alkaloids, flavonoids, saponins, coumarins and emodin's etc. using the standard procedure.

1) Test for Tannins

a) 2ml extract was added to 1% lead acetate a yellowish precipitate indicates the presence of tannins.

b) 4ml extract was treated with 4 ml FeCl**3** formation of green color indicates the presence of condensed tannin

2) Test for Saponin

5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponin.

3) Test for Anthocyanin

2 ml of aqueous extract is added to 2 ml of 2N HCl & NH**3**, the appearance of pink red turns blue violet indicates presence of Anthocyanin.

4) Test for Coumarin

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates Coumarins.

5) Test for Emodin's

2 ml of NH**4**OH and 3 ml of benzene was added to extract appearance of red color indicates presence of emodin's.

6) Test for Alkaloids

A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following test.

a) Wagner test: Filtrate was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.

b) Hager's test: Filtrate was treated with Hager's reagent, presence of alkaloids confirmed by the yellow-colored precipitate.

7) Test for Proteins

a) Xanthoproteic test: Extract was treated with few drops of concentrated HNO**3** formation of yellow indicates the presence of proteins.

8) Test for Amino acids

a) Ninhydrin test: To the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of blue color indicates the presence of amino acid.

9) Test for Diterpenes

a) Copper acetate test: Extract were dissolved in water and treated with 10 drops of copper acetate solution; formation of emerald green color indicates presence of diterpenes.

10) Test for Phytosterol

a) Salkowski's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H**2**SO**4** and shakes, allow standing, appearance of golden red indicates the positive test.

11) Test for Phenol

a) Ferric Chloride test: Test extract were treated with 4 drops of Alcoholic FeCl**3** solution. Formation of bluish black color indicate the presence of Phenol

12) Test for Phlobatannins

Deposition of red precipitate when aqueous extract of each plant sample is boiled with 1% Aqueous HCl was taken as evidence for presence of Phlobatannins.

13) Test for Leucoanthocyanin

5 ml of isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in color indicates the presence of Leucoanthocyanin.

14) Test for Cardial Glycosides

a) Keller-Killani Test: Plant extract treated with 2 ml glacial acetic acid containing a drop of FeCl3. A brown color ring indicates the presence of positive test.

15) Test for Flavonoids

a) Alkaline reagent test: Extract was treated with 10 % NaOH solution, formation of intense yellow color indicates presence of Flavonoids.

b) NH4OH test: 3 ml of extract were 10 % NH**4**OH solution development of yellow fluorescence indicates positive test.

Minimum inhibitory concentration (by Pour plate method):

A) Preparation of fresh bacterial cultures

Table No.1: Composition of Macconkey's Media

Composition of MacConkey's media (for *E.coli*) Ingredients	Quantity
MacConkey's broth	0.8 gm
Agar agar (type 1)	0.8 gm
Distilled water	20 ml

1. The ingredients given in formula were weighed and dissolved in distilled water in conical flask.
2. This solution is boiled with continuous stirring, until clear solution is obtained.
3. Sterilized by autoclaving at temperature 1210 C, pressure 15 psi for 15 min.
4.Sterilized Mac Conkey's media and Vogel Johnson agar transferred aseptically into separate hard glass test tubes previously sterilized, plugged with cotton and kept in slanting position.
5.When it is cooled and solidified, the bacterial cultures from existing slants were inoculated on these slants. (E.coli was inoculated on MacConkey's media slant and S.aureus was inoculated on Vogel Johnson agar slant.)
6. Kept in incubator for 24 hrs.
7. These test tubes with fresh bacterial cultures of E.coli and S. aureus then kept in refrigerator for further use.

B) Preparation of nutrient agar

Table No. 2: Composition of Nutrient Agar

Composition of Nutrient agar Ingredients	Quantity
Nutrient broth	6.5 gm
Agar agar (type 1)	7.5gm
Distilled water	500 ml

Procedure

1. The ingredients given in formula were weighed and dissolved in distilled water in conical flask.
2. Boiled to dissolve the agar and sterilized by autoclaving at temperature 1210 C, pressure 15 psi for 15 min.

C) Sample preparation

Table No.3: Sample Preparation

	Concentration	Extract of	Quantity of extract	Quantity of sterile water
1	200 mg/ml	Leaf	1.2 gm	6 ml
2	200 mg/ml	Stem	1.2gm	6 ml
3	500 mg/ml	Leaf	3 gm	6 ml
4	500 mg/ml	Stem	3 gm	6 ml

Weighed the extracts as per the above formula and dissolved in sterile water in different test tubes.

D) Preparation of bacterial suspension[10]

i. Preparation of Nutrient broth

0.26 gm of Nutrient broth is dissolved in 20 ml of Distilled water in a conical flask and boiled to dissolve, sterilized by autoclaving at temperature 1210 C, pressure 15 psi for 15 min.

ii. Preparation of bacterial suspension (in Aseptic conditions)

1 ml of sterile nutrient broth is transferred into a previously sterilized test tube and the one loopful of bacterial culture of E.coli from the slants transferred into the test tube and the turbid solution is formed. Similar procedure is carried out for S. aureus in different test tube.

E) Preparation of Petri plates with extract (by Pour plate method)

In this process two different concentrations were taken as follows:

1) 200mg and 500mg

i) 200 mg/ml and 500 mg/ml

Freshly the bacterial suspension was prepared inoculating one loop full of bacterial culture into 1 ml of sterilized nutrient broth. (For E.coli as well as S.aureus)

For leaves extract

a) For E.coli:

All the Petri plates and hard glass test tubes were cleaned & sterilized in hot air oven. The working area was made aseptic. 6 hard glass test tubes were taken and labeled as 1-6. (3 for 200mg/ml and another 3 for 500mg/ml) .Then 15 ml of nutrient agar is filled in all hard glass test tubes. One loop full of E.coli suspension was transferred into the third hard glass test tube and serial dilutions are made with second and first test tube. Repeat for remaining 3 test tubes. 1 ml of leaves extract solution having concentration 200/500 mg/ml was transferred into each test tube. Then it is poured into 6 separates labeled Petri-plates and kept in incubator for 24 hr and bacterial growth was observed.

a) For S.aureus:

Similar procedure was carried out using bacterial suspension of S.aureus instead of E.coli.

For Stem extract

a) For E.coli:

6 hard glass test tubes were taken and labelled as 1-6. (3 for 200 mg/ml and another 3 for 500mg/ml) then 15 ml of nutrient agar is filled in all hard glass test tubes. One loop full of E.coli suspension was transferred into the third hard glass test tube and serial dilutions are made with second and first test tube. Repeat for remaining 3 test tubes. 1 ml of Stem extracts solution having concentration 200/500 mg/ml was transferred into each test tube. Then it is poured into 6 separates labelled Petri plates and kept in incubator for 24 hr and bacterial growth was observed.

b) For S. aureus:

Similar procedure was carried out using bacterial suspension of S. aureus instead of E.coli.

F) Preparation of petriplates without extracts

a) For E.coli:

3 Previously sterilized hard glass test tubes were taken and labelled as 1, 2 and 3. Then 15 ml of nutrient agar is filled in all the three hard glass test tubes. One loop full of *E.coli* suspension was transferred into the third hard glass test tube and serial dilutions are made with second and first test tube. Then these are poured into 3 separates previously sterilized and labelled Petri plates and kept in incubator for 24 hr and bacterial growth was observed.

b) For S. aureus:

Similar procedure was carried out using bacterial suspension of *S. aureus* instead of *E.coli.*

5.2.8 Antimicrobial assay (By well diffusion method)

For antibacterial assay two drugs were used as standard such as Amoxicillin and Amikacin.

Amoxicillin for *E.coli* and Amikacin for *S. aureus*

Preparation of plates for assay

a) For E.coli:

25 ml of nutrient agar was poured into previously sterilized hard glass test tube and inoculated with 1 loop full of bacterial suspension of E.coli. This then poured into previously sterilized Petri plate aseptically and kept aside to cool and solidify. After agar is solidified 3 wells are formed. Standard drug Amoxicillin (500mg/ml), aq. extract of leaves (500 mg/ml) and aq. extract of stem (500

mg/ml) are filled in the wells and kept in incubator for 24 hr and results were observed.

b) For S.aureus:

25 ml of nutrient agar was poured into previously sterilized hard glass test tube and inoculated with 1 loop full of bacterial suspension of S.aureus. This then poured into previously sterilized petriplates aseptically and kept aside to cool and solidify. After agar is solidified 3 wells are formed. Standard drug Amikacin (500 mg/ml), aq. extract of leaves (500mg/ml) and aq. extract of stem (500mg/ml) are filled in the wells and kept in incubator for 24 hr and results were observed.

Results and Discussion

Collection and drying of plant materials: The Tridax Procumbens plant was collected and the leaves and stems were washed and dried

Figure No.2: Dried Leaves of Tridax Procumbens

Figure No.3: Dried Stems of *Tridax Procumbens*

Identification and Authentication of plant material

The herbarium sheet of dried plant specimen of Tridax Procumbens was prepared and authenticated at Botanical survey of India, Pune, by D. L. Shirodkar, Botanist, and Voucher specimen no. BSI/WRC/Iden.Cer. /2022/0104220016824. Figure 5 shows the authentication certificate.

Figure No.4: The Herbarium Sheet of Tridax Procumbens Plant

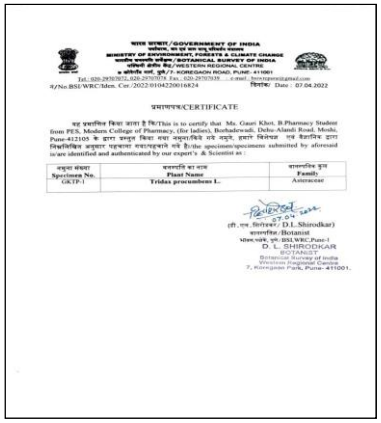

Figure No.5: Authentication Certificate of Tridax Procumbens

Grinding of plant materials

The dried leaves and stems of Tridax Procumbens were grinded to get the fine powder.


Figure No.6 and 7: Powder Characteristics

Study of Tridax Procumbens Leaves and Stem

Organoleptic Evaluation of Powdered Plant Materials

The powder of leaves of Tridax Procumbens have green color and characteristic odour and the powder of stem have yellowish green color and characteristic odour as shown in Figure 6.

Table No. 4: Colour and Odour of Powdered Plant Material

Sr. No.	Powder characteristics	Leaves powder	Stem powder
1	Color	Green	Characteristic
2	Odour	Yellowish green	Characteristic

Physicochemical parameters of powdered plant materials

a) Ash Value

Ash value indicates the purity of plant material. Total ash value, acid insoluble ash value and water-soluble ash value are given in table no.5

Table No. 5: Ash Value of Powdered Plant Material

Sr. no.	Powder characteristics	Leaves powder (%)	Stem powder (%)
1	Total ash	19	22.83
2	Acid insoluble ash	2.5	5.2
3	Water soluble ash	4.7	3.3

Total ash value, acid insoluble ash value and water-soluble ash values were found in standard range. These indicated the purity of powder of leaves and stem of Tridax Procumbens.

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b) Loss on drying

The moisture content of a drug should be less so as to prevent its decomposition due to chemical drug and microbial attack

Table No. 6: LOD of Tridax Procumbens

Sr. no.	Powder characteristic	Leaves powder %	Stem powder %
1	Loss on drying	13	15

From the above it was found that leaves and stems powder of Tridax Procumbens contains less moisture. So, it prevents decomposition of powder.

Extraction of powdered drug

30 g of Tridax Procumbens leaves powder and stem powder was extracted with distilled water by using Soxhlet extraction assembly. After Soxhlet extraction, % yield, color and consistency was reported in table 7.

Table No. 7: % Yield and Appearance of Aqueous Extract

Sr.no.	Extract of	Solvent	Colour	Consistency	% Yield w/w
1	Leaves	Distilled water	Greenish	Semisolid sticky mass	10
2	Stem	Distilledwater	Brown	Semisolid sticky mass	11

Preliminary phytochemical tests of aqueous extract

Phytochemical screening of aqueous extracts of leaves and stem was carried out by using standard procedures and it was found that it may consist of following groups of chemical constituents as shown in table 8.

Table No. 8: Preliminary Phytochemical Tests of Leaves and Stem Extract of Tridax Procumbens

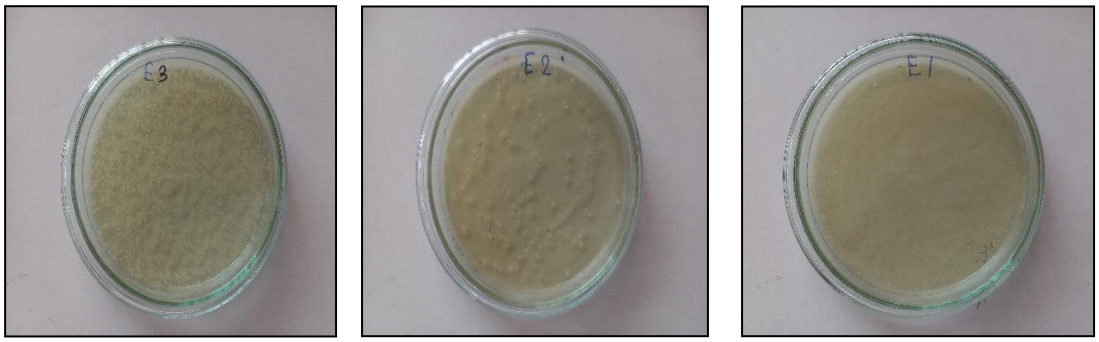
Sr. no.	Test for	Aq. extract ofStem	Aq. extract of leaves
1	Tannins a. Lead acetate b. Ferric chloride	+	+
2	Saponins		+
3	Anthocyanin	-	+
4	Coumarins	+	+
5	Coumarins	+	+
6	Emodins		-
7	Alkaloids Wagner's test, Hager's test	+	+
8	Proteins- Xanthoproteic test	+	+
9	Amino acids- Ninhydrin test	+	+
10	Diterpenes- Copper acetate test	-	+
11	Phytosterol- Salkowski test	-	+
12	Phenol	+	+
13	Phlobatannins	+	+
14	Leucoanthocyanin	-	-
15	Cardial glycosides- Kellar-Killiani test	+	+
16	Flavonoids a) Alkaline reagent test b) NH4OH test c) Zn test	 + - -	 + + -

Minimum inhibitory concentration (by pour plate method)

MIC was obtained by serial dilution method.

Agar Plates without extracts

i) E.coli without aq. extract:



(a) (b) (c)

Figure No. 8: Growth pattern of *E.coli*. (a), (b), and (C) shows plates labelled as E3, E2, and E1respectively.

(Where, E= E.coli)

E3 shows more growth as compared to E1 and E2.

ii) S.aureus without aq. extract:

(a) (b) (c)

Figure No. 9: Growth pattern of *S.aureus*. (a), (b), and (C) shows plates labeled as SA3, SA2, and SA1respectively (Where, SA= S.aureus)

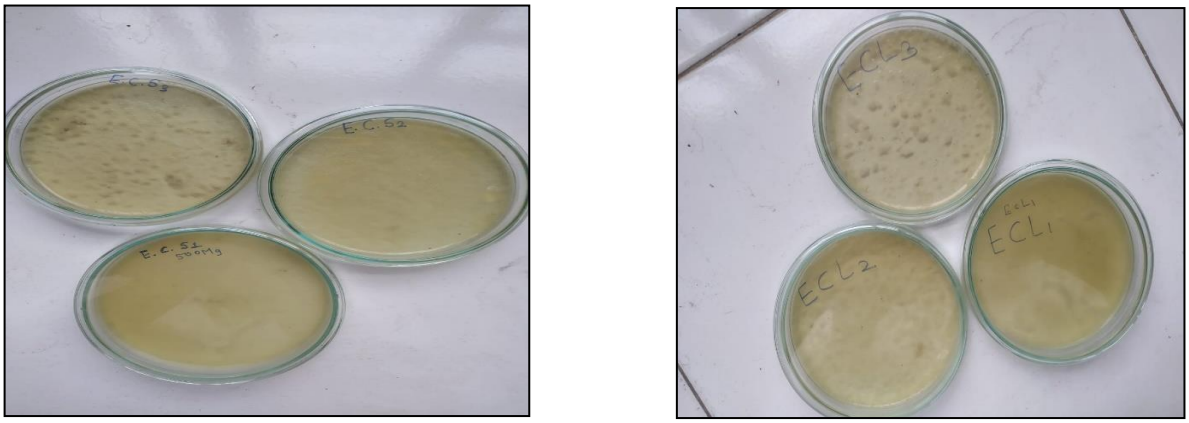
SA3 shows more growth as compared to SA1 and SA2.

Determination of MIC

MIC of aq. extracts of stem and leaves of Tridax Procumbens for E.coli and S.aureus were determined. 500mg/ml concentration of leaves and stem extract was taken and agar plates were prepared for E.coli and S.aureus. These plates were incubated for 24 hr and growth was observed as follows:

A. Aq. extract of concentration 500mg/ml

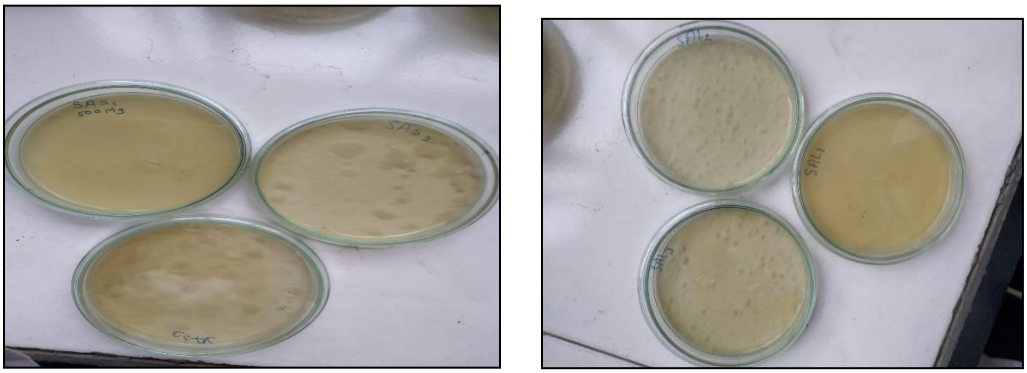
i) E.coli with aq. extract 500mg/ml concentration



(a) **(b)**

Figure No 10: The growth of *E.coli* in the presence of (a) Stem extract (500mg) & (b) Leaves extract (500 mg) of *Tridax procumbens*.

ii) S.aureus with aq. extract 500mg/ml concentration



(a) **(b)**

Figure No. 11: The growth of *S.aureus* in the presence of (a) Stem extract (500mg) & (b) Leavesextract (500 mg) of *Tridax Procumbens*.

Antimicrobial assay (well diffusion method)

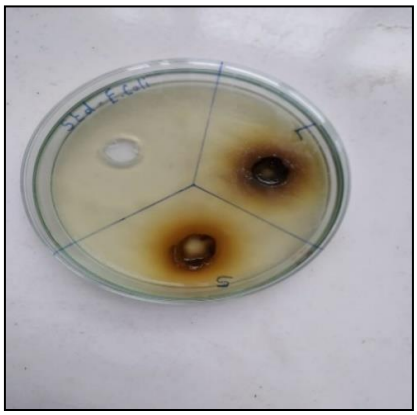
Antimicrobial assay was carried out by using well diffusion method. Standard drugs used as control were Amikacin and Amoxicillin for *S.aureus* and *E.coli* respectively.

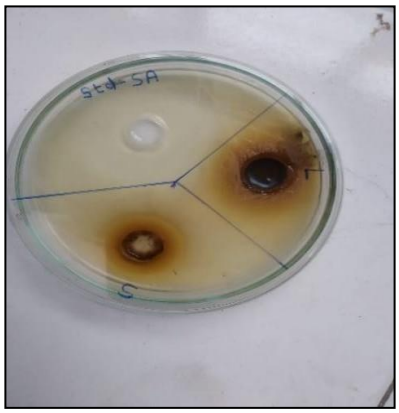
The plates after incubation for 24 hr were observed and zone of inhibition is measured. Fig 6.11 shows the zone of inhibition and the results are shown in table 9.

Table No. 9: Antimicrobial activity of aq. extracts of *Tridax Procumbens* against *E.coli* and *S.aureus*

Extract	Zone of inhibition (mm)	
	E.coli	*S.aureus*
S	24	30
L	12	16
Control	48 (Amoxicillin)	44 (Amikacin)

(Where, S= Stem extract, L= Leaves extract, Control= STD i.e. Amoxicillin for *E.coli* and Amikacin for *S.aureus;* the dose of extracts and control was 500 mg





(a) **(b)**

Figure No.12: Zone of inhibition of aq. extracts of stem and leaves of Tridax Procumbens. (a) For E.coli & (b) for S.aureus

Conclusion

In present study, aq. extracts of leaves and stem part of Tridax Procumbens were compared for antimicrobial activity. Firstly, all organoleptic and physicochemical properties of leaves and stem powder were evaluated and it shows that drug is pure and having required constituents sufficiently. Aq. extract of leaves powder and stem powder was prepared separately using Soxhlet extraction technique. Both the extracts were evaluated for physiochemical screening using standard procedures. Aq. extract of leaves shows presence of tannis, saponins, anthocyanin, coumarins, alkaloids, proteins, amino acids, diterpenes, phytosterol, cardial glycosides, phlobatannins and flavonoids. Aq. extract of stem shows presence of tannins, coumarins, phenol, cardial glycosides and

flavonoids. Both extracts were primarily evaluated for antimicrobial activity against E.coli and S.aureus by selecting dose of 500 mg. Finally, antimicrobial assay was performed for both extracts (500mg) against E.coli and S.aureus by well diffusion method using Amoxicillin and Amikacin as standards respectively. Antimicrobial assay shows that aq. extract of stem part is more effective against S.aureus than E.coli; also Leaves aq. extract shows antimicrobial activity against S.aureus and E.coli.

From present studies we can conclude that aq. extract of stem and leaves of Tridax Procumbens having antimicrobial activity and stem extract is more effective against S.aureus as compared to leaf extract.

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