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Antimicrobial Activity of Leaves of Tridax Procumbens Linn

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ABSTRACT: In the present work I have extract and evaluate microbiological effect of tridex procumbens. In which we had used various solvent (water, hydro ethanol and ethyl acetate) for extraction, from which I got satisfactory extract results in ethyl acetate and hydro ethanolic solvent. Actor extraction I had used both dried extract of tridax procumbens for evaluation of its microbiological activity by Cup-plate method and serial dilution method as well as compared with phenol coefficient method by rideal-walker coefficient. I have found antimicrobial activity e hydro ethanol and ethyl acetate extract of tridax procumbens between 250-300 µg/ml. I haven't found any antimicrobial activity in water extract. The hydro ethanolic and ethyl acetate extracts of tridax procumbens inhibited the growth of all selected bacterial species (Escherichia coli, Staphylococcus aureus and Lactobacillus) but their effectiveness is different. The ethyl acetate extract where more effective than the hydro ethanolic extract in in Cup-plate method and serial dilution method adopted. The test antimicrobial samples of tridax procumbens compared with standard reference phenol by phenol coefficient method. The rideal-walker coefficient and phenol coefficient is less than 1 therefore both test disinfectant (T. Procumbens Linn) is less effective than phenol.

KEYWORDS: Tridax procumbens, Phenol coefficient test, Cup-plate method, Liquid dilution method, Extract

I. INTRODUCTION

[1].Tridax procumbens is a widely spread hispid, procumbent herb, usually found as a weed. T. procumbens is perennial in nature with flowering-fruiting throughout the year. T. procumbens is commonly called as ‘Jayanti-veda’ in Sanskrit, Tikki-kasa/'Ghamra'in Hindi and ‘Wild daisy’, ‘Mexican daisy’ and ‘Coat buttons’ in English based on the appearance of the flower. The scientific name is ‘Tridax procumbens’. The generic name is derived from the Greek, meaning ‘summer eating', implying that it was a summer vegetable.

[1].T. procumbens belongs to the kingdom: Plantae, sub-kingdom: Tracheobionta, division: Magnoliophyta–Dicotyledons, class: Magnoliopsida, sub-class: Asteridae, order: Asterales, family: Asteraceae, genus: Tridax L. and species: procumbens.

[2].Many ancient traditions including the Ayurveda, Siddha and the Unani systems of medicine have advocated the use of several herbal preparations like plant juices and extracts for diseases including infectious ones. 74% of the plant- derived medicines have a modern indication that correlates with their traditional, cultural and sometimes ancient uses. Hence, traditional medicine is an important source for the development of novel chemotherapeutic agents which are less toxic and more economic.

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II. AIM AND OBJECTIVE

NEED FOR THE STUDY:-

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. The essential values and uses of some plants have been worked out and published, but many of them remain unexplored to date. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to discover their medicinal properties. Tridax procumbens (L.) is a spreading annual herb found throughout India but unfortunately it is one of the neglected plants. Hence, the present review aims to open new avenues for the improvement of medicinal use of Tridax procumbens (Compositae) for various ailments and to bring the anti-diabetic medicinal plant to the scientists' notice, and raise awareness and add value to the resource. This research attempts to highlight the available literature on Tridax procumbens (L.) with respect to ethno botany, chemical constituents and summary of various pharmacologic activities.

In India, all over the population maximum peoples are poor. They cannot purchase the medicine. But to recover their wounds and injury they use mixture of various plants leaves, fruit and other unorganized and organized part of plants. But they don't know their exact activity of specific single plant, so that's why I need to study of that plant, for their antimicrobial property. Many peoples don't understood the antimicrobial activity of tridax procumbence.

AIM: The aim of the present research was to study the Antimicrobial Activity of Leaves of Tridax Procumbens Linn

OBJECTIVES OF THE STUDY:

The purpose of the present study is:

1. Collection of the different plants and authentication.
2. Extraction of the required active constituents from the selected plants.
3. To carry out the antimicrobial study of various extract in a different concentration.

A. Cup-plate or cylinder plate method
B. Liquid dilution method or Test tube method
C. Phenol coefficient method

III. EXPERIMENTATION

Collection of medicinal plant

The leaves of Tridax procumbens were collected from local areas and college campus of Maharashtra institute of pharmacy, betala (Bramhapuri), Maharashtra, India. The collected T. procumbens were transported to the laboratory for identification and stored for further studies. It was shade dried for 5 days and powdered using a mixer grinder. The leaf powder was used for the extraction of phytochemicals.

METHODOLOGY

Materials used

All the chemicals and reagents used were of analytical grade provide by collage purchase from samar chemicals includes ethyl acetate, ethanol, bees wax, agar agar powder (bacteriological), and also from himedia includes Nutrient agar etc.

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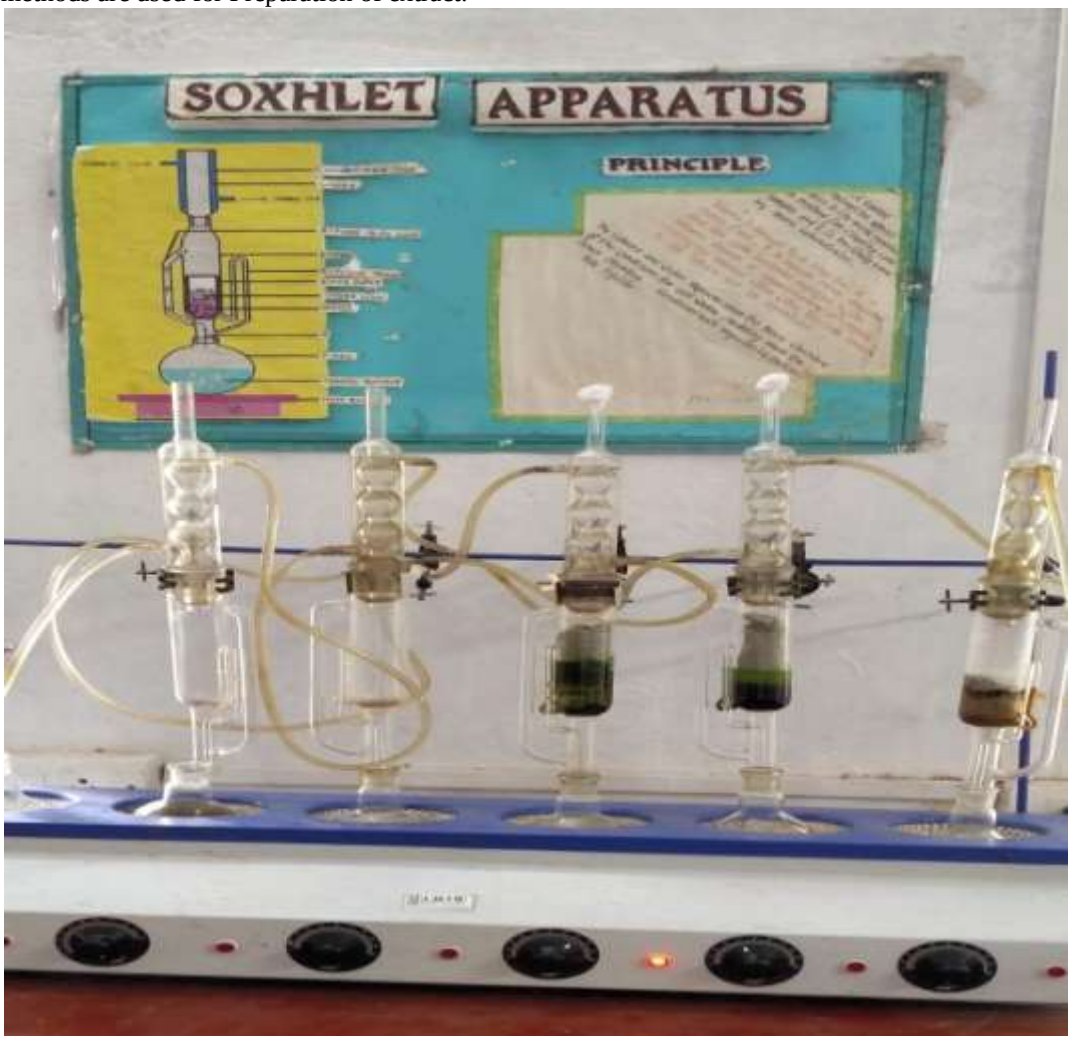
Instruments used

Soxhlet apparatus, mixer grinder, beaker, stirrer, Electric water bath, funnel, tripods stand, incubator, test tubes, petri plates, autoclave, Nephelometer Turbidity Digital Meter, heating mental, laminar air flow.

PREPARATION OF EXTRACT

Three methods are used for Preparation of extract.



> Put accurately weight 11.27 g of the phytochemicals containing sample (under filter paper bag) into extraction tube > Attach the extraction tube with flask containing solvent > Attach a condenser unit with the extraction tube and run the water > Fix the soxhlet apparatus on heating mental and heat the flask containing solvent at 80 to 100* C > The solvent starts to evaporated and falls in the extraction tube after condensed > Continued this process till for 24 hours > Discontinue the process and take out the extract > Again this extract put on electronic water bath for evaporation > Discontinue the process after solvent evaporated and collect sample.

A. Aqueous extract (leaves of tridax procumbens)

> Assembled the apparatus> Filled the round bottom flask with solvent (120 ml of water) > Put accurately weight 11.27 g of the phytochemicals containing sample
(Under filter paper bag) into extraction tube.

B. Ethyl acetate extract (leaves of tridax procumbens)

Assembled the apparatus > Filled the round bottom flask with solvent (90 ml of ethyl acetate) > Put accurately weight 6.49 g of the phytochemicals containing sample (under filter paper bag) into extraction tube > Attach the extraction tube with flask containing solvent > Attach a condenser unit with the extraction tube and run the water > Fix the soxhlet apparatus on

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heating mental and heat the flask containing solvent at 45 to 70* C > The solvent starts to evaporate and falls in the extraction tube after condensing > Continued this process till for 24 hours > Discontinue the process and take out the extract > Again this extract put on electronic water bath for evaporation > Discontinued the process after solvent evaporated and collect sample.

C. Hydro-ethanolic extract (leaves of tridax procumbens)

Assembled the apparatus > Filled the round bottom flask with solvent [Mixture of Ethanol (30ml) and water (110ml)] > Put accurately weight 11.58 g of the phytochemicals containing sample (under filter paper bag) into extraction tube > Attach the extraction tube with flask containing solvent > Attached a condenser unit with the extraction tube and run the water > Fix the soxhlet apparatus on heating mental and heat the flask containing solvent at 80 to 100* C > The solvent starts to evaporate and falls in the extraction tube after condensing > Continued this process till for 24 hours > Discontinue the process and take out the extract > Again this extract put on electronic water bath for evaporation > Discontinued the process after solvent evaporated and collect sample.

After that all the extracts were mixed in appropriate quantity (Table 02)

Antimicrobial study

A. Cup-plate method

Dissolved 50 mg of extract sample in 50 ml sterile water > From this-take 1 ml and dilute to 100ml of sterile water > Use stock solution to prepare 1, 2, 3 and 4 units/ ml and standard stock solution > Prepared a solution or unknown as a given sample of antibiotic in the same solvent as that of standard solution and diluted it till the level of standard preparation of the antibiotic > Sterilize the antibiotic assay medium by autoclave and prepare petri plates and laminar air flow > Spread the test microorganisms on the surface of Petri plates by spread plate technique > By using flame sterilized cork borer, prepared four cup in a plate keeping adequate distance from each other > Standard and test dilute antibiotic solution depending upon size are added in each labelled cavity of plates>Transfer all the plates in which for proper diffusion at antibiotic sample at 4 °C for one to two hours > Incubated all the plates in incubator at 37* C for one day > and another day I seen the zone of inhibition.

B. Liquid dilution method or Test tube method

Use a series of test tubes which contain a double strength medium and are labelled as shown in Table 13.9. In the first tube (un-inoculated), inoculum is not added which is used for checking the sterility of the medium. All other in an eleven test tubes, inoculum (3 to 4 drops) is added to reach the final concentration of microorganisms is 105 to 106 cells/ml. In all test tubes, test chemical is added ranging from 0.5 to 5 ml except in the uninoculated and control tube. The second tube (control) is used to check the suitability of the medium for growth of the test microorganism and the viability of the inoculum. The final volume (10 ml) in all test tubes is adjusted by using sterile water. The contents of all test tubes are properly mixed and incubated at 37°C for 2 to 3 days. After incubation, all test tubes are examined for the growth in the form of turbidity and the results are recorded and minimum inhibitory concentration is calculated. It is also necessary to conduct a preliminary experiment to determine the approximate range (test solution) which would be suitable for the test

Table: 01. Determination of MIC by liquid dilution method

Tube number	Volume of double strength medium (ml)	Volume of test chemical (ml)	Volume of sterile water (ml)
0 (uninoculated)	5	0.0	5
0' (control)	5	0.0	5
1	5	5.0	0.0
2	5	4.5	0.5
3	5	4.0	1.0
4	5	3.5	1.5
5	5	3.0	2.0
6	5	2.5	2.5
7	5	2.0	3.0
8	5	1.5	3.5

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9	5	1.0	4.0
10	5	0.5	4.5

C. Phenol coefficient method

In the phenol coefficient method a test chemical is rated for its microbicidal property with reference to phenol under identical conditions. In this test similar quantities of microorganisms are added to rising dilutions of phenol and of the disinfectant to be tested. In this method Escherichia coli, Staphylococcus aureus and Lactobacillus organisms are used.

The phenol coefficient test includes:
1. Rideal-Walker test (RW test)

Phenol coefficient test is suitable for testing disinfectants miscible with water and which exert their antimicrobial action in a manner similar to that of phenol. The phenol coefficient of test disinfectants may be calculated by Rideal-Walker test that use Rideal-Walker broth and Lactobacillus as the sensitive microorganism.

Different dilutions of the test disinfectant and phenol are prepared and 5 ml of each dilution is inoculated with 0.5 ml of the 24 hours broth culture of the organisms. All tubes (disinfectant + organisms and phenol + organisms) are placed in a 17.5°C water bath. Subcultures of each reaction mixture are taken and transferred to 5 ml sterile broth after 2.5, 5, 7.5 and 10 min. The broth tubes are incubated at 37°C for 48 to 72 hours and are examined for the presence or absence of growth.

A typical result is shown in Table 02. The Rideal-Walker coefficient of the test disinfectant is then calculated (as per results shown in Table 02) as follows:

IV. RESULTS AND DISCUSSION

Extraction results

After extraction of leaves of tridax procumbens we have obtained appropriate quantity (Table 02)

Table: 02-Extract Mixture

Sr. no.	Constituent	Percent (%)
A	Aqueous extract	27.05 %
B	Ethyl acetate extract	10.01 %
C	Hydro-ethanolic extract	25.99 %

Antimicrobial study results

A. Cup-plate method

Observed the zone of inhibition around the cavity. Measure the diameter of zone of inhibition using antibiotic zone reader and in recorded in the observation table

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A. Ethyl acetate extract B. Hydro-ethanolic extract

Table No. 03: Observation table for cup-plate method

Organisms	Minimum inhibition concentration (Diameter of zone of inhibition)		
	Aqueous extract (µg/ml)	Ethyl acetate extract (µg/ml)	Hydro-ethanolic extract (µg/ml)
Staphylococcus aureus	-	250 (12.1 mm)	350 (12.4 mm)
Escherichia coli	-	250 (10.8 mm)	300 (11 mm)
Lactobacillus	-	250 11.4 mm)	350 (11.7 mm)

B. Liquid dilution method or Test tube method

After incubation, all test tubes are examined for the growth in the form of turbidity and the results are recorded and minimum inhibitory concentration is calculated. It is also necessary to conduct a preliminary experiment to determine on Nephelometer Turbidity Digital Meter the approximate turbidity range (test solution) which would be suitable for the test.

1. for ethyl acetate extract

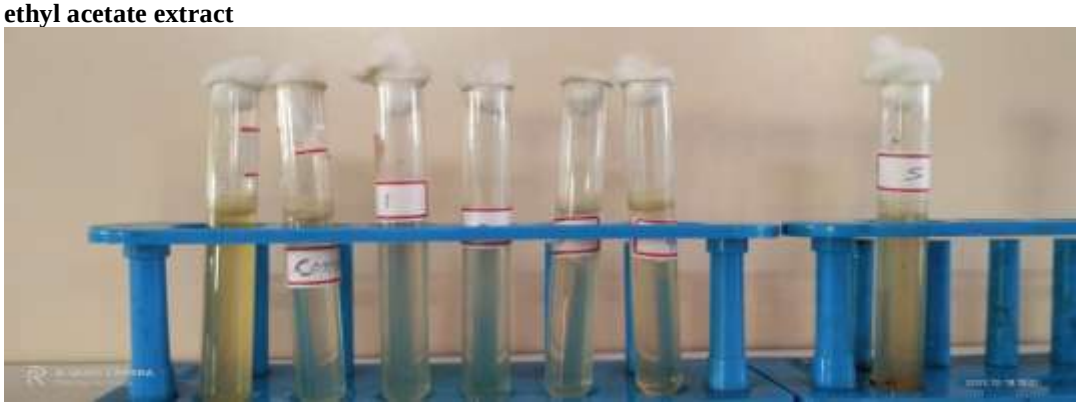

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Table 04: Result of MIC by liquid dilution method with their concentration

Tube number	Concentration (µg/ml)	Terbidometry readings
0 (uninoculated)	0	012
0' (control)	0	045
1	50	041
2	100	035
3	150	023
4	200	020
5	250	014
6	300	012
7	350	011
8	400	012
9	450	010
10	500	010

2. for hydro ethanolic extract

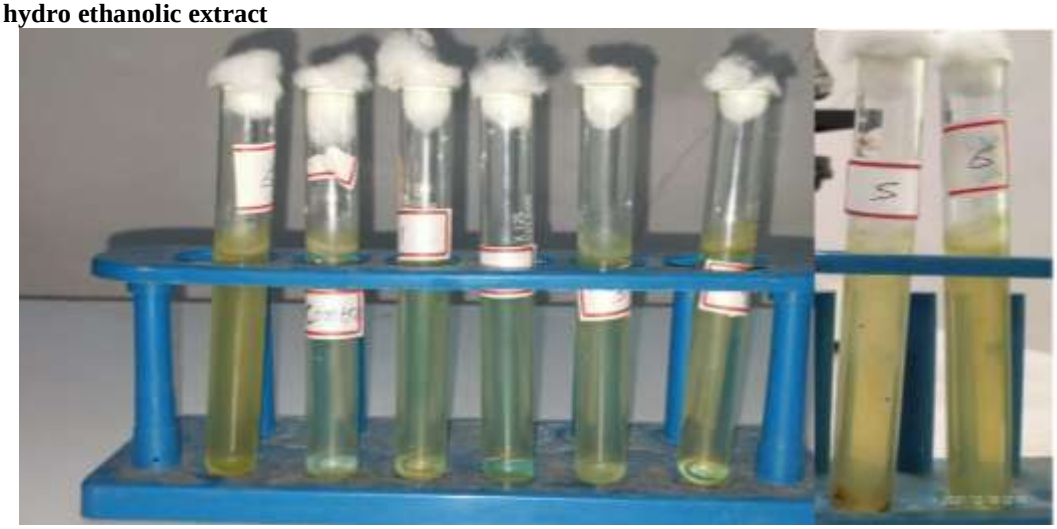

Table 05: Result of MIC by liquid dilution method with their concentration

Tube number	Concentration (µg/ml)	Terbidometry readings
0 (uninoculated)	0	011
0' (control)	0	042
1	50	038
2	100	033
3	150	025
4	200	020
5	250	014
6	300	012
7	350	011
8	400	010
9	450	011
10	500	009

C.Phenol coefficient method

The rideal-walker coefficient of the test disinfectant is then calculated (as per results shown in table 04) as follows:

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Table 06: Determination of rideal-walker coefficient

Disinfectant	Dilution	Time interval for sub-culture (min.)			
		2.5	5	7.5	10
Test Disinfectant (ethyl acetate extract)	1:40	-	-	-	-
	1:60	+	-	-	-
	1:80	+	+	-	-
	1:100	+	+	+	+
Test Disinfectant (hydro ethanolic extract)	1:40	+	-	-	-
	1:60	+	+	-	-
	1:80	+	+	+	-
	1:100	+	+	+	+
Phenol	1:80	+	-	-	-
	1:100	+	+	-	-
	1:120	+	+	+	-
	1:140	+	+	+	+

(+ = growth; - = no growth)

Calculation for ethyl acetate extract:

$$\text{R. W. Coefficient} = \frac{\text{Dilution of disinfectant killing in 7.5 but not in 5 min.}}{\text{Dilution of Phenol killing in 7.5 but not in 5 min.}}$$

$$= 80/100$$

$$= 0.8$$

The rideal-walker coefficient and phenol coefficient is less than one therefore test disinfectant (T. Procumbens Linn) is less effective than phenol.

Calculation for hydro ethanolic extract:

$$\text{R. W. Coefficient} = \frac{\text{Dilution of disinfectant killing in 7.5 but not in 5 min.}}{\text{Dilution of Phenol killing in 7.5 but not in 5 min.}}$$

$$= 60/100$$

$$= 0.6$$

The rideal-walker coefficient and phenol coefficient is less than one therefore test disinfectant (T. Procumbens Linn) is less effective than phenol.

V. SUMMERY & CONCLUSION

In the present work I have extract and evaluate microbiological effect of tridex procumbens. In which we had used various solvent (water, hydro ethanol and ethyl acetate) for extraction, from which I got satisfactory extract results in ethyl acetate and hydro ethanolic solvent. Actor extraction I had used both dried extract of tridax procumbens for evaluation of its microbiological activity by Cup-plate method and serial dilution method as well as compared with phenol coefficient method by rideal-walker coefficient. I have found antimicrobial activity e hydro ethanol and ethyl acetate extract of tridax procumbens between 250-300 µg/ml. I haven't found any antimicrobial activity in water extract. The hydro ethanolic and ethyl acetate extracts of tridax procumbens inhibited the growth of all selected bacterial species (Escherichia coli, Staphylococcus aureus and Lactobacillus) but their effectiveness is different. The ethyl acetate extract where more effective than the hydro ethanolic extract in in Cup-plate method and serial dilution method adopted. The test antimicrobial samples of tridax procumbens compared with standard reference phenol by phenol coefficient method. The rideal-walker coefficient and phenol coefficient is less than 1 therefore both test disinfectant (T. Procumbens Linn) is less effective than phenol. Result from the present study is possibly giving insight on the reason for this age long practice.

This investigation has opened up the possibility of the use of this plant in drug development for human consumption for the treatment of wound infection and various diseases.

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