

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF TRIDAX PROCUMBENS LINN WHOLE PLANT ETHANOLIC EXTRACT ON DIFFERENT PATHOGENS AND ITS PHYTOCHEMICAL SCREENING

Patil Vishin Ashish* and Nitave Sachin Annasaheb

Dr. J. J. Magdum Trust's, Anil Alias Pintu Magdum Memorial Pharmacy College,
Dharangutti, Tal: Shirol, Dist: Kolhapur, Maharashtra.

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*Correspondence for Author

Patil Vishin Ashish

Dr. J. J. Magdum Trust's,
Anil Alias Pintu Magdum
Memorial Pharmacy
College, Dharangutti, Tal:
Shirol, Dist: Kolhapur,
Maharashtra.

ABSTRACT

The ethanolic extract of *Tridax procumbens*, linn (Asteraceae) (commonly known as Coat Buttons) were evaluated for antimicrobial activity and antifungal activity. The dried powder of *Tridax procumbens* (leaves, stems, roots and flowers) containing chemical constituent procumbent was extracted and the activity was studied. Various concentration (5 mg/ml, 10 mg/ml and 15 mg/ml) of ethanol were evaluated to study the activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus flavus*, *Candida albicans*. The antibacterial activity was measured by agar well diffusion method and antifungal activity by disc diffusion method. *Tridax procumbens* (15 mg/ml) showed maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (18mm) and minimum against Gram negative bacteria *Escherichia coli* (8mm).

Tridax procumbens (15mg/ml) showed maximum antifungal activity towards *Aspergillus flavus* (12mm) and *Candida albicans* (4mm). The results showed significant activity of *Tridax procumbens* and suggesting its use as natural antimicrobial agent. Ampicillin was used as standard antibacterial drug Normal saline solution and alcohol was used as control to study antimicrobial activity and Amphotericin B was used as antifungal drug to study antifungal activity. The results of present study indicated that ethanolic extract of *Tridax procumbens* linn shows has potent antimicrobial and antifungal activity.

KEYWORDS: *Tridax procumbens*, Antibacterial, Antifungal activity, *S.aureus*, *E.coli*, *P.aeruginosa*.

INTRODUCTION

Man always been surrounded by countless microorganisms. The disease producing microbes are playing a very important role in human life. Pathogenic microorganisms are always trying to develop resistance to the various antimicrobial agents used for their control. Therefore, the chemotherapy of communicable diseases has proved to be a continuous great effort. Scientists are forever in exploring of new antimicrobial agents to run the ever increasing menace of the microbes. Thus it is of overriding importance for the microbiologists to develop new resistant strains. Therefore, medicinal plants are gifts of nature to cure limitless number of diseases among human beings.^[1]

Tridax procumbens linn is commonly known as 'Ghamra' in Hindi and 'Dagadi Pala' in Marathi. It is a weed found throughout India. A hispid, procumbent herb with woody base sometime rooting at the node, up to 60 cm high.^[2] Leaves are ovate-lanceolate 2 to 7 cm and lamina pinnatisect, sometimes three lobed, flowers in small, long peduncled heads. It is commonly used in Indian traditional medicine as anticoagulant, antifungal and insect repellent, in bronchial catarrh, diarrhea and dysentery. Moreover it possesses wound healing activity and promotes hair growth. The leaf gel possesses antiseptic, insecticidal and parasiticidal properties.^[3] The plant also shows various pharmacological activities like Immunomodulatory, Antidiabetic, Anti hepatotoxic & Anti-oxidant, Anti-inflammatory, Analgesic, and marked depressant action on respiration.^[4]

Vernacular Names

English- Coat Buttons and Tridax Daisy, Hindi- Ghamra, Sanskrit- Jayanti Veda, Marathi-Dagadi Pala, Telugu- Gaddi Chemanthi, Tamil- Thata poodu, Malayalam-Chiravanak, Spanish- Cadillp Chisaca, French- Herbe Caille, Chinese- Kotobukigiku.^[5]

MATERIAL AND METHOD

Collection And Authentication Of Plant Material

Fresh whole plant (leaves, stems, roots and flowers) of *Tridax procumbens* were collected from R.K.Nagar, Kolhapur, premises and authenticated by Dr.Miss. K.R.Datar (Head of Dept of botany) Deccan Education Society Willingdon college, Sangli. After authentication, fresh plant was collected in bulk, washed under running tap water, dried under shade for a period of 7 days and then pulverized in mechanical grinder to obtain coarse powder. The dried powder was stored in airtight bottles.

Chemicals

Methanol, Dextrose, Peptone, Agar, Distilled water, Ampicillin, Amphotericin B, Barium chloride dehydrate, Sulphuric acid.

Fungal strains

The bacterial and fungal strains for the study were obtained from Govt. Medical college, (Microbiology and bacteriology department). The fungal strains and bacterial strains used in the study are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus flavus*, *Candida albicans*.

Ethanol extract of *Tridax procumbens*

The coarse powdered material (each 100 gm) was soaked in 95% ethanol (500ml) by Soxhlet technique for continuous 72 hours. The extract was evaporated to dryness until dry mass is obtained. The yield obtained was 0.483%.^[6]

ASSESSMENT OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY^[7,8]

1. Preparation of Inocula

From fungal cultured slants, several colonies were transferred to 5ml of sterile distilled water. It is mixed for some seconds to ensure homogeneity and further diluted to match the turbidity with 0.5 McFarland standard solution corresponding to (1×10^6) CFU/ml).

2. Preparation of samples

Tridax procumbens linn alcoholic sample solutions were prepared at 5mg/ml, 10mg/ml, 15mg/ml concentrations in alcohol ii) Amphotericin B was taken as standard antifungal drug to study antifungal activity and Ampicillin was taken as standard antibacterial drug to study antibacterial activity.

3. Disc diffusion method

- i) Sabouraud Dextrose agar is prepared as fungal media and sterilized.
- ii) All glasswares, filter disc, petriplates, extract dilutions were sterilized in autoclave.
- iii) In aseptic technique, using sterile swab a bacterial lawn is made on sterile petri plates from microbial inoculum suspension. Swab is made in one direction by rotating plate at 90°.
- iv) Sterile filter discs of 6mm diameter were impregnated with about 0.1ml/disc of each extract dilution solution and placed on agar plate in aseptic condition.

v) Plates are incubated at 28^oc-30^oc for 2 days. Alcohol, Sterile distilled water are kept as control. After 2 days zone of inhibition was measured. In case of alcoholic dilutions of *Tridax procumbens linn* the zone of inhibition of alcohol is subtracted from control alcohol zone of inhibition.

3. Agar Well Diffusion Method

The antibacterial activity of *Tridax procumbens* was evaluated by using agar well diffusion method. Bacterial cultures are mixed in nutrient agar medium and poured in Petriplates. Wells or cups of 5mm size were made with sterile borer into agar plates containing the bacterial inoculums. 2mg of crude *Tridax procumbens linn* was completely dissolved in 2ml of Ethanol 95%. Antibacterial activity was measured at different concentrations of extract ranging from 5,10,15 mg/ml drugs *Tridax procumbens linn*. The zone of inhibition of alcohol is subtracted from control alcohol zone of inhibition. Ethanol 95% served as control and antibiotic Ampicillin served as standard.

RESULT

Tridax procumbens (15 mg/ml) showed maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (18mm) and minimum against Gram negative bacteria *Escherichia coli* (8mm). *Tridax procumbens* (15mg/ml) showed maximum antifungal activity towards *Aspergillus flavus* (12mm) and *Candida albicans* (4mm).

Table 1: Effect of *Tridax procumbens Linn* extract on growth of bacteria *in vitro*. Zone of inhibition (mm)

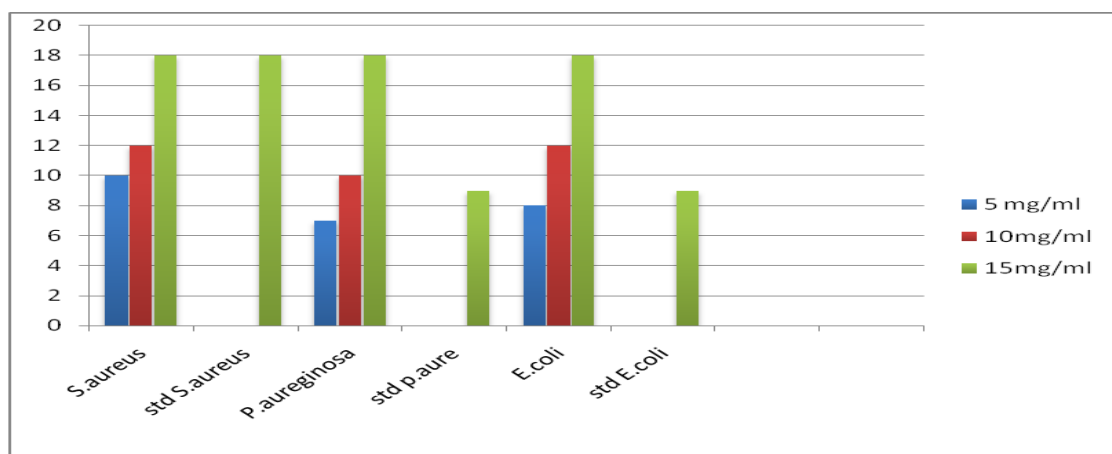
Sr. No.	Extract Concentration	Zone of Inhibition in mm of diameter		
		<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
<i>Tridax procumbens linn</i>				
1.	5mg/ml	10 mm	7 mm	8 mm
2.	10mg/ml	12 mm	10 mm	12 mm
3.	15mg/ml	18 mm	16 mm	18 mm
4.	Ampicillin (15mg/ml)	18 mm	9 mm	9 mm

Table 2: Effect of *Tridax procumbens Linn* extract on Pathogenic fungi Zone of inhibition (mm)

Sr. No.	Extract Concentration	Zone of Inhibition in mm of diameter	
		<i>Aspergillus flavus</i>	<i>Candida albicans</i>
<i>Tridax procumbens linn</i>			
1.	5mg/ml	8 mm	4 mm
2.	10mg/ml	10 mm	8 mm
3.	15mg/ml	12 mm	10 mm
4.	Amphotericin B (15mg/ml)	8 mm	14 mm

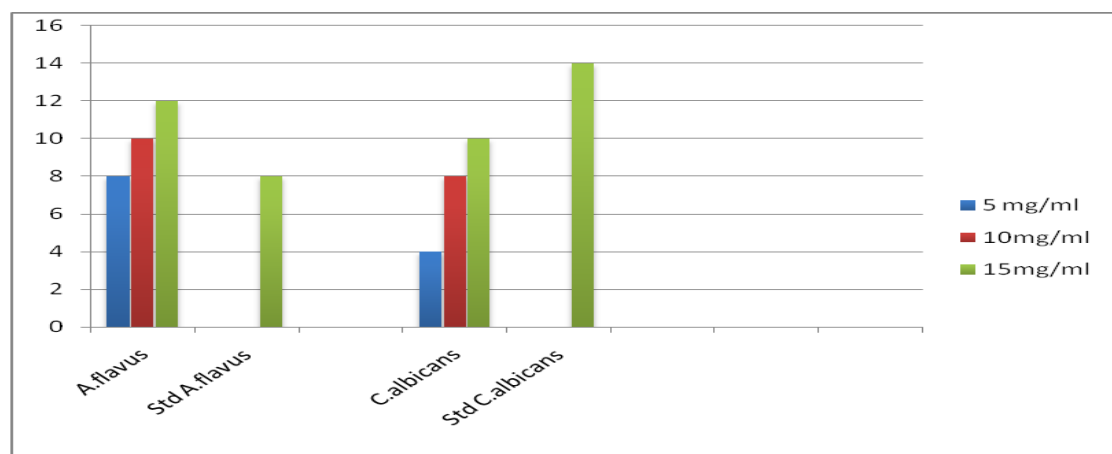
Table 3: Phytochemical Evaluation of *Tridax procumbens* linn^[9]

CHEMICAL TESTS	RESULT	CHEMICAL TESTS	RESULT
<u>Test For Carbohydrates</u> A. Benedicts Test B. Fehling's Test C. Molisch's Test	Positive Positive Positive	<u>Test For Tannins</u> A. 5% Ferric chloride B. Acetic acid test C. Dil. KMnO ₄ Test	Positive Positive Positive
<u>Test For Steroids</u> Salkowaski test	Positive	<u>Test For Flavonoids</u> A. Lead acetate test B. NaOH + Dil. acid	Positive Positive
<u>Test For Alkaloids</u> Dragendroff's test Wagner's test Mayer's test	Positive Positive Positive	<u>Test for Glycosides</u> Borntrager's test	Positive



X axis: concentration in mg/ml Y axis: zone of inhibition in mm

Figure 1: Effect of *Tridax procumbens* Linn extract on Pathogenic bacteria Zone of inhibition (mm).



X axis: concentration in mg/ml Y axis: zone of inhibition in mm

Figure 2: Effect of *Tridax procumbens* Linn extract on Pathogenic fungi Zone of inhibition (mm)



Figure 3: Effect of alcoholic extract of *Tridax procumbens* linn on *Aspergillus Flavus*

DISCUSSION

The occurrence of antibacterial and antifungal substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines of it can be the base for the development of a medicine, a natural blueprint for the development of a drug. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The results showed significant activity of *Tridax procumbens* linn and suggesting its use as natural antimicrobial agent. The result of present study indicated that ethanolic extract of *Tridax procumbens*, linn shows potent antimicrobial and antifungal activity.

CONCLUSION

From the recent study it is concluded that, as dose of the *Tridax procumbens* increases the antimicrobial activity as well as antifungal activity increases. From the observations it clearly indicate that *Tridax procumbens* has potent antimicrobial activity as well as antifungal activity but it act by dose dependent manner.

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