

Antimicrobial Activity of Methanolic Extract of Flowers of *Tridax Procumbens*

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Abstract: Herbs are natural remedies for the disease with higher safety profile and efficacy. India is gifted with varieties of large number of medicinal herbs because of variety of climatic conditions and seasons favorable for growth of many species of plants. Amongst the large number of herbal drugs existing in India, very few have been studied systematically so far. *Tridax procumbens* (L) is a highly valuable drug and is one of the essential ingredients in the most of the compound preparations included in Ayurvedic literature. It is well known for number of pharmacological activities like hepatoprotective activity, anti-inflammatory, wound healing, Antidiabetic activity, hypotensive effect, immunomodulating property, bronchial catarrh, dysentery, diarrhea and to prevent falling of hair, promotes the growth of hair, and antimicrobial activity against both gram-positive and gram-negative bacteria. The leaf juice shows antiseptic, insecticidal and parasiticidal properties, against conjunctivitis and is used also to check hemorrhage from cuts, bruises and wounds insect repellent. It is also used as bioadsorbent for chromium. Here we try to make attempt for focusing on antimicrobial activity of *Tridax procumbens* flowers. Their antibacterial activities were evaluated in vitro against clinical bacterial isolates. In effort to identify novel bacterial agents, this study was initiated to evaluate the antimicrobial properties of *Tridax procumbens* flowers extract against *E. coli* and *Staphylococcus aureus* by using disc-diffusion assay. The flower plant extracts demonstrated good antimicrobial activity against these bacteria tested with inhibition zones. The minimal inhibitory concentration (MIC) values of extract against the tested bacteria were found to 67.3 mg/ml and 48.20 against *E.coli* and *Staphylococcus aureus* respectively. The methanolic extract of flowers exhibited a pronounced activity against *E. coli* and *Staphylococcus aureus*. The minimum inhibitory concentration was found in methanolic Extracts of *Tridax procumbens* against test organisms.

Keyword: *Tridax procumbens*, Antimicrobial activity, *E.coli*, *Staphylococcus aureus*

Introduction

With 'Herbal Renaissance' happening all over the globe, medicinal herbs are staging a phenomenal comeback. Ethno botanical information estimates that more than 6000 higher plant species forming about 40% of the higher plant diversity, are used in its codified and folk healthcare traditions. [1] As sources of biologically active molecules and blue prints for the development of modified derivatives with enhanced activity and or reduced toxicity, plant-derived drugs form an important segment of the modern pharmacopoeia. Ethnopharmacologists, micro- biologists and botanists are combing the earth for such natural treasures and developing novel chemotherapeutic agents which are less toxic and more economic. [2]

A major stumbling block to the successful management of infectious diseases has been the propensity with which microorganisms are able to develop resistance to routinely used antibiotics. The development and spread of multidrug resistant superbugs especially in the hospital environment, continues to be a burning global issue due to the indiscriminate and irrational use of antibiotics. [3]

The use of the medicinal herbs for curing disease has been documented in history of all civilization. It was concluded that plants contain active principles, which are responsible for the curative action of the herbs. The isolated active constituent of medicinal herbs and after

testing some found to be therapeutically active.^[4] One such plant known to be associated with various pharmacological properties including antimicrobial ones is *Tridax procumbens*, belonging to the daisy family, are found perennially in various tropical and subtropical regions as well as mildly temperate regions worldwide.^[5] Listed as a weed and a pest plant, it has been known by several names including *Tridax* daisy in English, Jayanti veda in Sanskrit, Ghamra in Hindi, Thata poodu in Tamil.

Reports state that the leaf juice can be used to cure fresh wounds, and also as a hair tonic.^[5] A few reports have focused on the immense potential of this plant which has antimicrobial, wound healing, anti-inflammatory and immunomodulatory properties.^[6] ^[7] However, there is a paucity of reports on its effect on nosocomial and multidrug resistant pathogens. Hence this study was carried out to compare the antibacterial activity of aqueous and ethanolic extracts of the leaves of *T. procumbens* and to study the efficacy of the extracts against community acquired and nosocomial human pathogens. To the best of our knowledge, the effect of *T. procumbens* against multidrug resistant nosocomial pathogens has not been studied so far. Hence it is worthy to do it.

Materials and Methods

Plant

The fresh flowers *Tridax procumbens* Linn. (Compositae) were collected in the months of march from the garden located in campus of Pataldhamal Wadhvani College Of Pharmacy, Yavatmal, Maharashtra state, India, and authenticated by the authority of botany department, Amravati University, Amravati.

Bacteria

Pathogenic strains of *Staphylococcus aureus* s, and *Escherichia coli* (ATCC) were obtained from pathology department from Sheri Vasantrya Naik Government Medical College Yavatmal, and were maintained on agar slant medium(HiMedia) at 4 °C for further experiments.

Culture Media

Types of media was required for carrying out this study, Nutrient agar (biolife) and Mueller-Hinton agar (HiMedia). Also methanol was used for extraction process. These media issue from pathology department from Shri Vasantrya Naik Government Medical College Yavatmal , and the solvent were issue from store room in pataldhamal wadhvani college of pharmacy yavatmal.

Antibiotics

Gentamycine antibiotic (Eye Drops) purchase potency 0.3% w/v in 10 ml and 1 ml contain 300 unit /ml purchased from Rukhamani medical store located in S.B.I Road manufactured by Allergan India pvt. Limited shows antibiotics potency.

$$\% \frac{w}{v} = \frac{\text{mass of solute}}{\text{volume of solution}} \times 100$$

1 ml contain 300 unit /ml

Therefore 10 ml contain 3000 unit /ml

40 unit = 1 ml

8 unit = 0.1 ml; 0.0125 ml= 1 unit

0.125 ml= 10 unit

Standard concentration of gentamycin is 10 µg/ml

Preparation of Plant Extract

Flowers of *Tridax procumbens* plant were collected and kept for shed drying for optimal period. After complete drying they were subjected for grinding into coarse powder by the mechanical means. The powdered flowers were kept for maceration in methanolic medium for 96 Hrs at room temperature and filtered [10].

Assessment of Activity

By using well diffusion method

The antibacterial activity was performed by agar well diffusion method. [8] In this method Mueller –Hinton (MH) agar petridishes is used (by using autoclave at 121°C for 15 min). The PH level of the agar was maintained between 7.2 and 7.4 by adding NaOH and HCl. MH agar at 37°C was inoculated with a MH broth culture of each bacterial species and poured over the agar plates to form a homogenous layer. Three to four well (holes) were made in the plates (of about 6mm diameter) using a sterile corn borer and plant extract was tested in triplicate with gentamycin (10µg/ml) well as a reference or positive control. [8] The plates were evaluated after incubation at 37°C for 24 hours after which the zone of inhibition around each was measured by using a scale in millimeters (mm). The ratio between the diameter of inhibition zone (mm) produced by plant extracts and the inhibition zone around the well with gentamycin (mm) was used to express antibacterial activity.

Results

Table 1: Phytochemical screening of Methanolic extract of flowers of *Tridax procumbens* Linn

Tests	Methanolic Extract
Carbohydrate	
Molish's	+ve
Fehling's	+ve
Benedicts	+ve
Cardiac glycoside	
Legal test	+ve
Saponin	
Foam test	-ve
Flavonoids	
Shinoda test	+ve
Lead acetate test	+ve

Table 2: Inhibition zone (mm) by Methanolic extract of flowers of *Tridax procumbens* against *E. coli* at different concentration

Species	Concentration	Zone of inhibition (mm) mean.
E. Coli	67.3 mg/ml	12.5 ± 1.378
	150 mg/ml	16.16 ± 0.7528
	Std 10µg/ml	18.83 ± 0.7528



Figure 2.1: MIC concentration of extract 67.3 mg/ml against *E.coli*

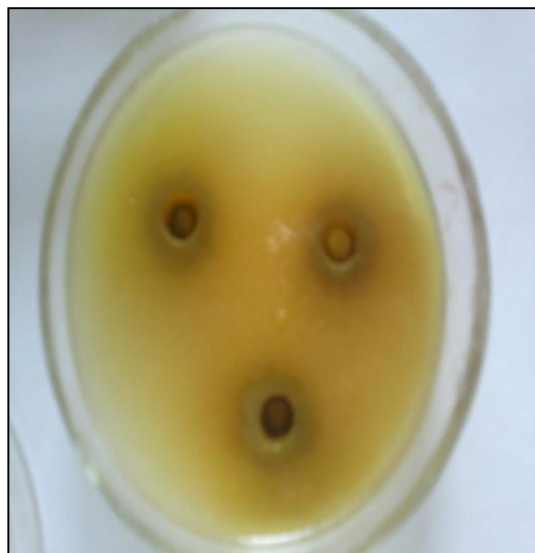


Figure 2.2: MIC concentration of extract 150 mg/ml against *E.coli*

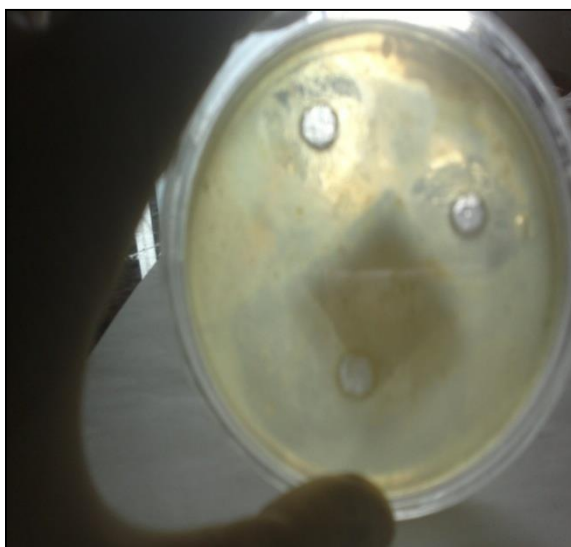


Figure 2.3: MIC of standard gentamycine 10µg/ml Against *E.coli*

Table 3: Inhibition zone (mm) of methanolic extract of flowers of *tridax procumbens* against *S. Aureus* at different concentration

Species	Concentration	Zone of inhibition in(mm) mean
S. Aureus	48.20 mg/ml	17.5 ± 0.7528
	100mg/ml	21.83 ± 0.7528
	Std 10 µg/ml	28.16 ± 1.169

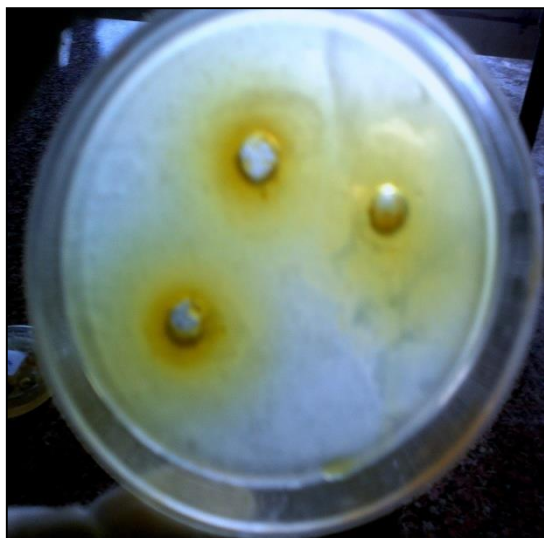


Figure 3.1: MIC concentration of extract 48.20 mg/ml against *S. Aureus*

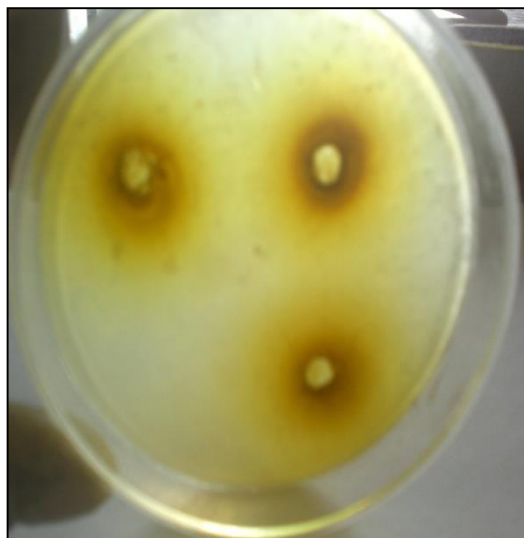


Figure 3.2: MIC concentration of extract 100 mg/ml against *S. Aureus*



Figure 3.3: MIC standard of gentamicin 10µg/ml Against *S. Aureus*

Table 1 the preliminary phytochemical screening of methanolic extract of flowers of *tridax procumbens* Linn revealed the presence of carbohydrate, glycoside, and Flavonoids.

Table 2 & figure 2.1, 2.2, 2.3 Shows the zone of inhibition by methanolic extract of flowers of *tridax procumbens* Linn. Methanolic extract is active at 67.3 mg/ml and 150mg/ml as compared to standard dose of gentamycine against *E.Coli*

Table 3 & figure 3.1, 3.2, 3.3 shows the zone of inhibition by methanolic extracts of flowers of *tridax procumbens* Linn. Methanolic extract is active at 48.20 mg/ml and 100mg/ml, as compared to standard dose of gentamycine against *S. Aureus*.

Discussion

Resistance in micro-organisms to many antimicrobial has resulted in morbidity and mortality from treatment failure and increased health care costs and increasing capability of microbes to develop multidrug resistance has encouraged search for new, safe and effective bioactive agents of herbal origin. It has been reported that *Tridax procumbens* medicinal plants have been used in the treatment against different diseases.^[9] Effect of methanolic extract on different micro-organisms i.e *S. aureus* and *E. coli*. The methanolic extract shows zone of inhibition in well diffusion method after incubation of plates for 24 hours at 37 °c. As per the results, *s. aureus* showed maximum zone of inhibition by methanolic extract compared with *E. coli*. On the basis of the antibacterial assay of this study *S. aureus* was found more (susceptible to the employed flowers extracts) than *E. coli*. The methanolic extract of flowers of *tridax procumbens* was evaluated for their MIC against *E. coli* and *S. aureus* at 67.3 mg/ml and 48.20 mg/ml respectively. The methanolic extract of *Tridax procumbens* showed significant antimicrobial activity against positive *Staphylococcus aureus* and on *E. coli* gram negative bacterial strains. Gram positive (*S. aureus*) bacteria and gram negative bacteria (*Escherichia coli*), showed a reduction in their growth on treatment with the different concentration of extracts of *Tridax procumbens*. The degree of inhibition was measured by the well diffusion method, reported that the more zone of inhibition in gram positive when compare with gram negative bacteria.

Conclusion

The methanolic extract of flowers of *Tridax procumbens* (L) has significant antibacterial activity against *S. Aureus* and *E. coli*. But *staphylococcus aureus* i.e. *gram positive* bacteria are more susceptible than *Escherichia coli* i.e. *gram negative bacterial*.

References

1. Akujobi C, Anyanwu BN, Onyeze C and Ibekwe VI: Antibacterial activities and preliminary phytochemical screening of four medicinal plants. *J Appl Sci* 2004; 7(3): 4328-4338.
2. Cowan, M.M. Plant products as antimicrobial agents. *Clin. Microbiol Rev.*, 12(4), 564-582. (1999).
3. Esimone CO, Adiutwu MV and Okonta JM: Preliminary antimicrobial screening of the ethanolic extract from the lichen *Usnea subfloridans* (L). *J Pharmaceutic Res Dev*; 3(2): 99-102. (1998).
4. Lipipun V, Kurokawa M, Suttisri R, Taweechotipatr P, Pramyothin P, Hattori M, et al. Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection in vitro and in vivo. *Antiviral Res*, 60(3), 175-80. (2003).
5. Mundada, S., Shivhare, R. Pharmacology of *Tridax procumbens* a Weed, Review. *International Journal of Pharm. Tech. Research*, 2(2), 1391-1394. (2010)
6. Pitout, J.D., Laupland, K.B. Extended spectrum beta lactamase producing Enterobacteriaceae, An emerging public-health concern. *Lancet Infect Dis.*, 8,159-66. (2008).
7. Racio, M.C., Rios, J.C., Villar, A. A review of some antimicrobial compounds isolated from medicinal plants. *Phytotherapy Res.*, 3(4), 117-125. (1989).
8. Suseela, L., Sarsvathy, A., Brindhya, P. Pharmacognostic studies on *Tridax procumbens* L. (Asteraceae). *Journal of Phytological Research*, 15(2), 141-147. (2002).
9. Taddei, A., Rosas, Romero, A.J. Bioactivity studies of extracts from *Tridax procumbens*. *Phytomedicine*, 7(3), 235-238. (2000).
10. Ved, D.K., Goraya, G.S. Demand and Supply of Medicinal Plants in India. National Medicinal Plants Board, New Delhi & FRLHT, Bangalore, India, ISBN, 978-81-211-0628-3, viii. (2007).