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# Evaluation and distribution of antibacterial potential in the aerial parts of wild *Tridax procumbens*

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## ABSTRACT

*Antibacterial activity of hexane, petroleum ether, chloroform and methanol extracts obtained from the aerial parts (leaf, flower and stem) of Tridax procumbens was tested against both gram positive (Staphylococcus aureus and Bacillus subtilis) and gram negative (Enterobacter aerogenes) bacteria using the agar well diffusion method. The susceptibility of the test bacteria varies with the types of solvents and plant parts used. The flower posses potent antibacterial activity with 20 mm of zone of inhibition against E. aerogenes in hexane extract whereas the leaf showed antibacterial activity in all the solvents used. The stem exhibited moderate inhibitory effect on the test bacteria. The potentiality of the leaf against the test bacteria as evaluated by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) indicated the presence of more active compounds in methanol than in hexane extracts. Chloroform extracts was found to be least active, whereas, petroleum ether possessed moderate effect on the test bacteria. These results may suggest the distribution of antibacterial potential in different aerial parts of T. procumbens that can be explored further for the isolation and charachterization of the compound agent in pharmaceutical industries.*

**Keywords:** Antibacterial, Antimicrobial, Asteraceae, Pathogens, *Tridax procumbens*

## INTRODUCTION

The large number of synthetic drugs produced from pharmaceutical industries from time to time has led to develop resistant microorganisms that become major global issue in the treatment of infectious diseases [1]. At present, there is an urgent and continuous need of exploration and

development of cheaper and effective new plant based drugs with better bioactive potential and least side effects. Antimicrobials of plant origin have been proved to be effective in the treatment of infectious diseases simultaneously with lesser side effects, which are often associated with synthetic antibiotics [2].

A large number of medicinal plants belonging to the Asteraceae family contained chemical compounds exhibiting antimicrobial properties. The family Asteraceae includes about 25,000 species, many of which are rich in secondary metabolites with biological activity [3]. Various studies have been carried out on some of Asteraceae plants, e.g. *Achillea* sp. [4], *Eupatorium* sp. [5], *Tridax* sp. [6] and *Ageratum* sp. [7]. The aerial parts of different species of the genus *Achillea* are widely used as a folk medicine due to numerous pharmacological properties, such as anti-inflammatory, antioxidant, antispasmodic and anti-hemorrhoidal [4]. Extract from natural dried leaves of *Eupatorium* sp. possess both antibacterial [5] and antimalarial [8] activity. Traditionally, *Ageratum* sp. is used as antidysenteric [7] and in the treatment of leprosy and purulent opthalmia [9]. *Tridax* sp. is commonly used in Indian traditional medicine as anticoagulant, hair tonic, insect repellent, anti-diarrhea, and anti-dysentery [6]. However, little attention has been given to antibacterial potential of different extracts of aerial parts of the *Tridax* sp.

Both gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Enterobacter aerogenes*) have been proved to be major causal organisms of various human infections such as food poisoning, nosocomial infections, wound infections and urinary tract infections and have been selected for the present study. Therefore, in the present investigation *Tridax procumbens* was selected to evaluate antibacterial potential of different plant parts against *Enterobacter aerogenes*, *Bacillus subtilis* and *Staphylococcus aureus* with a view towards further exploration as a source of new antimicrobials.

## EXPERIMENTAL SECTION

### *Plant material*

The whole plant of *Tridax procumbens* was collected locally from Kukrail forest, Lucknow, India. The plant was identified and stored in the form of herbarium in the Department of Biotechnology, Integral University as a specimen.

### *Preparation of plant extracts*

The aerial parts of the plant (leaf, flower and stem) were shade dried for five days. The plant material were finely ground and dried powder (25 g) of each part were extracted sequentially using soxhlet extractor with 250 ml of hexane, petroleum ether, chloroform and methanol separately in order to extract non-polar and polar compounds [10]. The crude extracts were then filtered through Whatman No. 1 filter paper and concentrated in vacuum at 40 °C using a rotary evaporator. The concentrated extracts were subsequently dried aseptically at room temperature.

### *Microorganisms and Growth conditions*

The test bacteria used, included *Bacillus subtilis, Enterobacter aerogenes and Staphylococcus aureus* were procured from National Chemical Laboratory (NCL) Pune, India. Bacterial strains were cultivated in Nutrient Broth. For antibacterial testing fresh inoculum was prepared for each

bacteria and were incubated at 37 °C for 24 h. The cells suspension was adjusted with nutrient broth to obtain turbidity comparable to that of McFarland 0.5 standard ($1.5\times10^8$ cells/ml).

**Antibiotic sensitivity testing**

Antibiotic sensitivity of bacterial strains was determined by the standard disc diffusion method of Bauer *et al.* [11] against a number of antibiotics such as Amoxicillin, Erythromycin, Chloramphenicol and Tetracycline having potency of 10 µg per disc.

***Determination of antibacterial susceptibility***

***(a) Agar well diffusion method***

The antibacterial assay of the extracts of aerial parts of *T. procumbens* were carried out by using agar well diffusion method [12]. 100 µl of diluted inoculum ($1.5 \times10^8$ CFU/ml) of test bacteria was swabbed over plates containing sterile Mueller Hinton agar (pH 7.2). Wells of 4 mm diameter were punched into the agar plate and filled with 40 µl of extract prepared in DMSO (50 mg extract / ml of DMSO). The plates were left for 30 min at room temperature to allow the diffusion of the extract and then incubated at 37 °C for 18 h. The inhibition zone was measured in millimeters. DMSO without extract was used as a control.

***(b) Micro dilution method***

Plant extracts showing significant antibacterial activity in agar well diffusion method was selected to determine the Minimum inhibitory concentration (MIC) by using micro-dilution bioassay [13]. Crude plant extracts were dissolved in DMSO to make stock solution of 100 mg/ml. 100 µl of each extract stock solution were two fold serially diluted with sterile nutrient broth in a 96-wells of microtiter plates for each bacteria. Thereafter, 100 µl inoculum ($1.5 \times10^8$ CFU / ml bacterial suspension) was added to each well. The microtiter plates were incubated at 37 °C for 24 h. Each extract was assayed in duplicate. 50 µl of 2 mg/ml p-iodo-nitrotetrazoleum chloride (INT) to each well was added and incubated at 37 °C for 30 minutes. The reddish-pink colour indicates growth of bacteria in the microtiter plate and clear wells indicates the inhibition by extract. The MIC values were taken as the lowest concentration of extract in the well that showed no color. The minimum bactericidal concentration (MBC) was determined by subculturing 50 µl from each well showing no apparent colour and the least concentration of extract showing no visible growth on agar plate was taken as MBC.

**Statistical analysis**

All data are given as the mean ± S.E. of three measurements. Statistical analysis was performed using spss version 10.

## RESULTS

In the present study, the antibacterial activity of the extracts (hexane, petroleum ether, chloroform and methanol) obtained from different parts (leaf, flower and stem) of *Tridax procumbens* was tested against *Bacillus subtilis, Enterobacter aerogenes and Staphylococcus aureus* [Figures 1-4].

Figure 1a, b and c shows the effect of various concentrations (0.25, 0.5, 1 and 2 mg/ well) of leaf, flower and stem hexane extracts on antibacterial activity against *B. subtilis*, *E. aerogenes* and *S. aureus*, respectively. All the tested bacteria responded differently against different extracts

and the effect of the extracts was concentration dependent. Maximum zone of inhibition (12 mm) was noticed with hexane extract of stem against *B. subtilis*, whereas, hexane flower and leaf extracts caused inhibition zone of 20 mm and 11 mm against *E. aerogenes* and *S. aureus*, respectively. The hexane extract of stem did not show any activity against *S. aureus* at any concentrations.

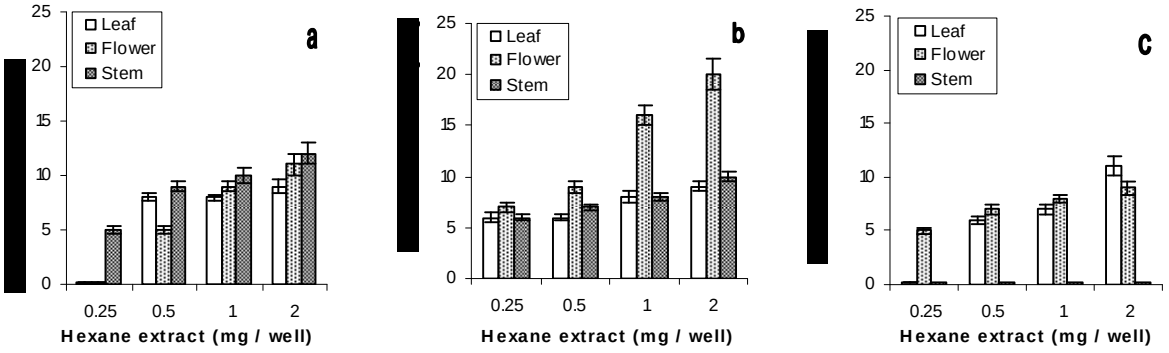


**Figure 1: Antibacterial activity of Hexane extracts of leaf, flower and stem of *Tridax procumbens* against *B. subtilis* (a), *E. aerogenes* (b) and *S. aureus* (c). Values are means ± SE with *n* = 3**

The petroleum ether extracts of leaf, flower and stem showed antibacterial activity against *B. subtilis*, *E. aerogenes* and *S. aureus* [Figure 2a, b and c]. The petroleum ether stem and leaf extracts exhibited 10 and 9 mm inhibition zones, respectively against *B. subtilis*. However, no activity was detected towards petroleum ether flower extract. *E. aerogenes* and *S. aureus* were found to be insensitive to the petroleum ether stem extracts. Whereas, both flower and leaf extracts showed 10 mm inhibitory zone against *E. aerogenes* and 7 and 10 mm inhibitory zones against *S. aureus*, respectively at high concentration of the crude extracts.

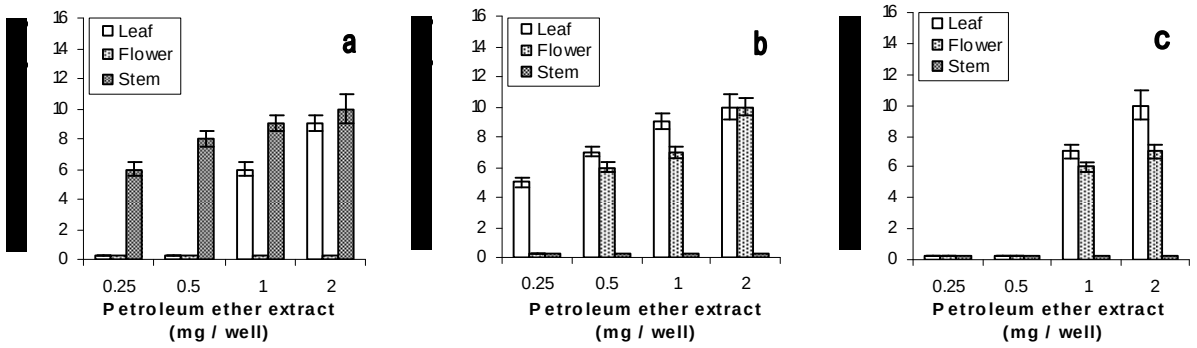


**Figure 2: Antibacterial activity of Petroleum ether extracts of leaf, flower and stem of *Tridax procumbens* against *B. subtilis* (a), *E. aerogenes* (b) and *S. aureus* (c). Values are means ± SE with *n* = 3**

The antibacterial activity of extracts of Chloroform leaf, flower and stem against *B. subtilis*, *E. aerogenes* and *S. aureus* is shown in figure 3 a and b. The chloroform extracts of all the aerial parts of the plant showed least sensitivity as 9 mm zone diameter against *B. subtilis* and 13 mm zone against *E. aerogenes* were recorded by chloroform leaf extracts, whereas, chloroform stem extract showed inhibition zone of 6 mm only in *B. subtilis*. While flower extracts did not show any activity for all the tested bacteria.

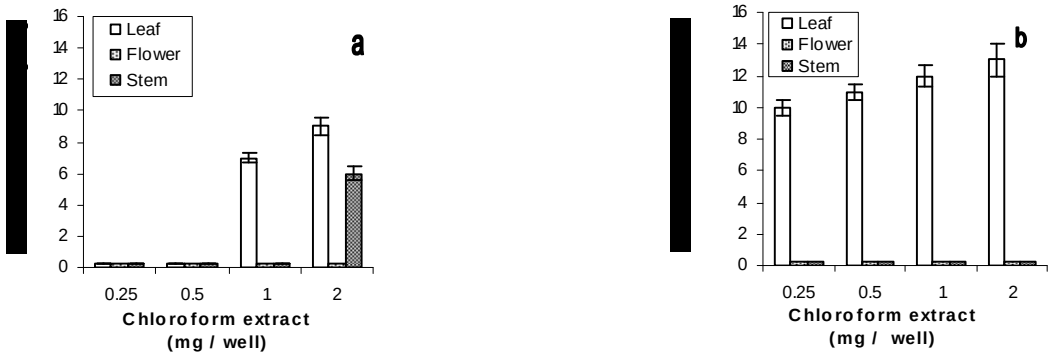


**Figure 3: Antibacterial activity of Chloroform extracts of leaf, flower and stem of *Tridax procumbens* against *B. subtilis* (a) and *E aerogenes* (b). Values are means ± SE with *n* = 3**

The methanolic extracts of leaf, flower and stem showed considerably high activity against *B. subtilis* as 13, 6 and 10 mm zones of inhibition, respectively were noticed. *E. aerogenes* and *S. aureus* were sensitive only to the methanol leaf extracts with zones of inhibition 11 and 7 mm, respectively [Figure 4a, b and c].

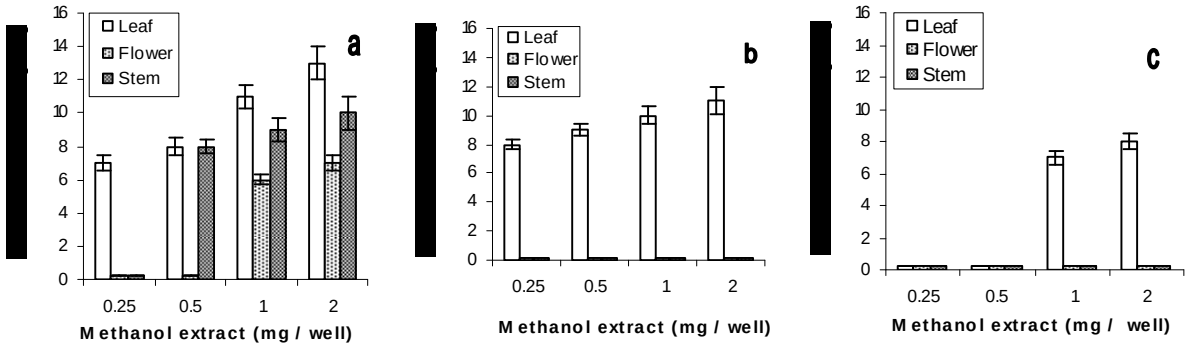


**Figure 4: Antibacterial activity of methanol extracts of leaf, flower and stem of *Tridax procumbens* against *B. subtilis* (a), *E aerogenes* (b) and *S aureus* (c). Values are means ± SE with *n* = 3**

**Table 1. Minimal inhibitory concentration and minimal bactericidal concentration of leaf extracts of *Tridax procumbens***

| Bacteria | Hexane extract | | Methanol extract | |
|---|---|---|---|---|
| | MIC (mg / ml) | MBC (mg / ml) | MIC (mg / ml) | MBC (mg / ml) |
| *Bacillus subtilis* | 2.5 | 5 | 1.25 | 2.5 |
| *Staphylococcus aureus* | 1.25 | 2.5 | 1.25 | 5 |
| *Enterobacter aerogenes* | 1.25 | 2.5 | 0.67 | 1.25 |

*MIC, Minimal inhibitory concentration; MBC, Minimal bactericidal concentration*

The hexane and methanol leaf extracts showed a significant antibacterial activity against all tested strains. Therefore, hexane and methanol leaf extracts were further selected for MIC and MBC assay [Table1]. Methanol and hexane leaf extracts were found to be highly active against

*E. aerogenes*, with MIC (0.67 mg/ml and 1.25 mg/ml) and MBC (1.25 mg/ml and 2.5 mg/ml) respectively, however, appreciable activity was observed against *B. subtilis*, with MIC (1.25 mg/ml and 2.5 mg/ml) and MBC (2.5 mg/ml and 5mg/ml), respectively. The MIC and MBC of methanol leaf extract was 1.25 mg/ml and 5 mg/ml, while, hexane leaf extract showed an MIC of 1.25 mg/ml and MBC of 2.5 mg/ml against *S. aureus*.

## DISCUSSION

The plant crude extracts generally, inhibit gram-positive bacteria rather than the gram-negative bacteria [14, 15] but from the present study the extracts of *Tridax procumbens* were equally susceptible to both gram-negative bacteria (*Enterobacter aerogenes*) and gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) [Figure 1, 2, 3 and 4]. These results are in concordance with those found by Bushra and Ganga [16]. The various crude extracts of *Eupatorium odoratum* and *Ageratum conyzoides* also showed the significant activity against both gram positive and gram negative bacteria [3].

The antibacterial activities of *Tridax procumbens* extracts were tested against three bacterial strains. The results showed promising antibacterial activity against the bacteria tested. Among these, hexane extract was found to have a more potent inhibitory effect followed by methanol extract while the other extracts have moderate effect [Figure 1 a, b, and c]. Radha *et al.* [17] studied the antimicrobial activity of different extracts (chloroform, ethyl acetate, methanol and water) of *Heliotropium marifolium* by standard dilution test using Muller Hinton Agar (MH) medium. Not only the solvents but the extracts of different plant parts showed variable degree of antibacterial activity against the test bacteria. Several researchers have reported that different parts of many plants such as flower, barks, stem and leaf etc. posses antimicrobial properties [18].

The hexane flower extracts gave best activity against *E. aerogenes* followed by stem and leaves extracts [Figure 1 b]. Similarly, Srinivasan *et al.* [19] investigated the potent antibacterial activity of hexane extracts of *Vicoa indica* (Asteraceae) leaves against *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholerae, Bacillus subtilis* and *Streptococcus pneumoniae*. Petroleum ether extract of *Tridax* sp. was equally active against the test bacteria [Figure 2 a, b and c]. Chloroform extract was less potent and only leaf extract was found effective against *E. aerogenes* and leaf and stem extracts were active against *B. subtilis* [Figure 3 a and b]. *S. aureus* was found to be insensitive against the chloroform extracts.

The screening of the chemical groups found in the powder material of this species as described by Salie *et al.* [20], showed the presence of tannins, saponin, triterpene and flavonoids. This activity may be attributed to the presence of the high concentration of secondary metabolites such as tannins and flavonoids, known to possess antimicrobial properties [21], in the different active extracts. Most antimicrobial active components that have been identified are not water soluble and thus organic solvent extracts have been found to be more potent [22]. Methanol extracts from leaf, stem and flower showed prominent antibacterial effect against *B. subtilis* whereas *E. aerogenes* and *S. aureus* were sensitive only with leaf extracts [Figure 4 a, b and c]. Polyphenolic compounds such as flavonoids, tannins and most other reported bioactive compounds are generally soluble in polar solvents such as methanol as reported by Nino *et al.* [23]. Ali *et al.* [24] reported that various phytochemical compound (alkyl esters, sterols,

flavonoids and pentacyclic triterpenes) as the major components of *T. procumbens*. The difference in susceptibility of various test bacteria towards the extracts as observed in the study could be due to the nature of the antimicrobial agents present in the extracts and their mode of action on the different test bacteria [25].

The preliminary results thus obtained are interesting evaluation of the potential antibacterial activity of *Tridax procumbens*. Further studies need to be carried out to define active principle of extracts from different aerial parts and their pharmacological actions.

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