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# Phytochemical Analysis and Antibacterial Activity of Active Extracts from *Tridax Procumbens* L. against Selected Pathogens

KEYWORDS: Tridax procumbens L., Antibacterial activity, Human pathogens, Secondary metabolites

**V. A. Kamble**
Department of Microbiology, Adarsha Science, J. B. Arts and Birla Commerce Mahavidhyalaya, Dhamangaon Railway, Dist- Amravati - 444 709 M.S. (India)

*** A. H. Moon**
Department of Microbiology, Adarsha Science, J. B. Arts and Birla Commerce Mahavidhyalaya, Dhamangaon Railway, Dist- Amravati - 444 709 M.S. (India). *Corresponding Author.

**ABSTRACT** *The present study deals with the antibacterial activity and phytochemical analysis of Tridax procumbens L. The extracts obtained from five solvents of different parts like leaf, stem, flower & roots were assayed using agar well diffusion method. All extracts of different parts responded variedly. The maximum zones of inhibition were measured for S. aureus (MTCC 96) as 15 mm & 14 mm by acetone root & methanol flower extracts respectively. The active extracts of methanol, acetone & ethyl acetate potentially exhibited the antibacterial activity for the B. subtilis (MTCC 554), E. coli (MTCC119), S. typhi (MTCC 734), K. pneumoniae (MTCC 109), S. aureus (MTCC 96) and S. paratyphi A (MTCC 735). The major phytochemicals such as alkaloids, phenols, cardiac glycosides, tannins and terpenoids were detected in the methanol extract in the optimum proportion. The study validates the use of medicinal plant for the infections caused by assayed pathogens.*

## INTRODUCTION

The potency of the antimicrobial activity of any plant species lies in its active phytochemical constituents. Therefore phytochemical screening is guiding key for determining the antimicrobial activity. The most active phytochemicals present in the plant are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952; Ali et al., 2001). These phytochemicals often termed as secondary metabolites of the plants. The pathogenic microorganisms like *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi A, Klebsiella pneumoniae* and others are recognized as the common human pathogens, infection occurs from these pathogens may sometime lead to complications. Due to developing resistance to any particular antibiotic, often results in treatment failure. Therefore searching the new antimicrobials is the matter of urgency to treat the infections. Many researchers were focused on the plant derived compounds as antimicrobial source, due to cheap, easily affordable, relatively less/ no side effects. From ancient time people relies on primary treatment from medicinal plants (Mayer et al., 2010, Zhang et al., 2004). The plant *Tridax procumbens* L. belongs to the family of *Asteraceae*. This plant is used from long time in Indian traditional medicine as anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and in dysentery. Moreover, it possesses wound healing activity for minor cuts, burn, small injuries and promotes hair growth (Saraf et al., 1991). Therefore the present study was intended to screen the phytochemicals of active extracts of *Tridax procumbens* L. & their antimicrobial activity against the potentially pathogenic microorganisms.

## MATERIALS & METHODS:

### Plant Materials

Different parts of *Tridax procumbens* L. (root, stem, leaf, flowers) were collected from different localities of Wardha, District of Maharashtra state. Collection was done during January 2012 to April 2012, from nearby areas like railway routes, gardens, and farms & washed thoroughly with distilled water. The cleaned plant parts are then allowed for the complete shade drying and then made to fine powder with a mechanical grinder and stored in an airtight container. The plant was authenticated in the Department of Botany, Adarsha Mahavidhyalaya, Dhamangaon (Rly).

### Extract Preparation

The shade dried plant material was subjected to Soxhlet extraction with ethanol, methanol, acetone, chloroform, and ethyl acetate. The dried powder (25 g) of each part was extracted sequentially using Soxhlet extractor with 250 ml of pure organic solvent for 24 – 48 hours separately. The crude extracts were then filtered through Whatman No.1 filter paper and concentrated at 40°C using a drier. The concentrated extracts were subsequently dried aseptically at room temperature. Extract was stored in sterile screw cap bottles under refrigeration condition at $4^0$ C prior to use for subsequent assays.

### Phytochemical analysis

Phytochemical examination was carried out of all extracts for detection of carbohydrate, cardiac glycosides, proteins, flavonoids, tannins, terpenoids, saponins, alkaloids, phenols, resins, phytosterols and fixed oils & fats as per standard methods (Harborne 1973, Trease 1989, Sofowara 1993).

### Antibacterial assay

For determining antibacterial activity, the standard test cultures of *Bacillus subtilis* (MTCC 554), *Listeria monocytogenes* (MTCC 657), *Proteus vulgaris* (MTCC 426), *Escherichia coli* (MTCC 119), *Salmonella enteric ser. typhi* (MTCC 734), *Proteus mirabilis* (MTCC 425), *Klebsiella pneumoniae* (MTCC 109), *Staphylococcus aureus* (MTCC 96) and *Salmonella enteric ser. paratyphi A* (MTCC 735) were used. The agar well-diffusion assay was used to determine the antibacterial assay (Laouer et al., 2009, Nair et al., 2005). The solidified plate was seeded with test bacterial suspension earlier matched with the 0.5 McFarland standards, a sterile cotton swab was dipped into the standardized inoculum and rotated firmly against the upper inside wall of the test tube to remove excess inoculum from the swab. Entire sterile and dried Mueller Hinton agar surface of the plate was streaked with the swab three times, by turning the plate $60^0$ between each streaking. Wells of 8 mm were

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cut into the agar using a sterile stainless steel borer and were filled with 100 µl of various extracts. The plates were incubated at 37 $^0$C for 24 hours. The zone of inhibition was measured using a zone reader and the results were noted by subtracting the zone of inhibition produced by pure solvent which was used as a control.

**RESULTS AND DISCUSSION**

Phytochemical analysis (Table 1**)** revealed the presence of most bioactive compounds in the methanol extract, ranging from low to moderate & high concentration. The phytochemical screening of different parts like leaf, stem, flower & root revealed the presence of alkaloids, flavonoids (catechins and flavones) and tannins. The major bioactive compounds detected were flavonoids, terpenoids, alkaloids, phytosterols, cardiac glycosides, phenols, resins and saponins. The least extraction of bioactive compounds was noted in the chloroform extract in very low concentration. The other solvents like acetone, ethyl acetate & ethanol also detected the bioactive components.

The secondary metabolites of the plant play a major role in confirming the antimicrobial activity. Alkaloids are nothing but alkali-like natural compounds found in both plants and animals. Many drugs and poisons are alkaloids in nature. The well-known are morphine, codeine, strychnine, nicotine and cocaine, etc. Terpenoids constitutes as the natural products whose structures are considered to consist into several isoprene units; therefore, these compounds are invariably termed as isoprenoids. The known examples are quassin, plant volatile compounds, essential oils, etc. Terpenoids are attributed for analgesic and anti-inflammatory activities and flavonoids have been reported to possess many useful properties like anti-inflammatory, estrogenic, antimicrobial, antiallergic, antioxidant, antitumor activity etc (Tania Da S. et al.,).

Our study finding supported the results of the other researchers. The plant was richly endowed with carotenoids and Saponins (Ikewuchi et al., 2009). The phytochemical analysis of the *Tridax procumbens* plant & its extract from various solvents were studied by different researchers (Patel et al., 2011, Sathya Bama S. et al., 2012, Nino et al., 2002, Tejaswini et al., 2011, Aniel Kumar O and L. Mutyala Naidu 2008).

**Antibacterial activity-**

The five extracts of the four parts of plant were tested against nine bacterial species. The extracts of the chloroform, ethyl acetate, acetone, ethanol and methanol responded to the bacterial species variedly (Table 2). The most inhibited bacterial species included the *S. aureus* (MTCC 96). The inhibition zone range of 8 mm to15 mm was noticed for *S. aureus* (MTCC 96). The highest zone of inhibition of 15 mm showed by acetone root extract. Acetone stem extract showed 13 mm zone, while leaves extract showed 12mm zone. Extraction in methanol solvent for flowers and roots revealed 14 mm and 13 mm inhibitory zones, respectively. Rizvi et al., (2011) reported the zone of inhibition of 11mm for *S. aureus* by hexane flower and leaf extracts of *Tridax procumbens.* They also reported 7 mm zone by methanol extract of leaf. Our study, showed the higher inhibitory zones against *S. aureus* by methanol and acetone extract.

The second most inhibited bacteria was *S. typhi* (MTCC 734), the zone range was noticed as 9 mm to 15mm. *S. typhi* (MTCC 734) showed maximum inhibitory zones in acetone extract as 15 mm against roots, 12mm against both flowers and leaves; and 11 mm against stems. Whereas, *S. paratyphi A* (MTCC 735) showed inhibitory zone range in acetone extract as 10 to 13 mm against different parts of the plant. Acetone extract also showed inhibitory spectra for *B. subtilis* (MTCC 554), the growth inhibitory zones was recorded as 14mm against stems, 13mm against flowers, 12 mm against roots and 11 mm against leaves. The methanol extract showed inhibitory zones against *E. coli* (MTCC119) as 10 mm for leaves, 11 mm for roots and 9 mm for both, stems and flowers of *Tridax procumbens*. For *P. vulgaris* (MTCC 426), methanol extract shown zones as 14 mm against flowers, 11 mm against leaves, 9 mm against roots, while stems not shown any inhibitory activity. Against *K. pneumoniae* (MTCC 109), zones of inhibition recorded from ethyl acetate extract were 12 mm for stem, 11 mm for root, 8 mm for flower, while leaves not shown any zone. Dhasarathan et al., (2011) reported less antimicrobial effect of ethanol, chloroform, water extract and higher antimicrobial action of butanol extract of *Tridax procumbens* against assayed pathogens. They reported the predominant inhibition zones of 38, 47, 42, 41 and 42 mm against *E. coli, B. subtilis, P. vulgaris, K. pneumoniae* and *S. aureus*, respectively of the phytochemicals extracted in butanol solvent. In present study, significant level of antimicrobial effects of acetone and methanol extracts for stem, flower and root parts of *Tridax procumbens* were recorded against tested pathogens. The less affected bacteria were *P. mirabilis* and *L. monocytogenes*. For *P. mirabilis* (MTCC 425) lowest zone were recorded in methanol extract as 8 mm by stems, leaves and flowers, while root extract produced 9mm zone. The lowest zone were shown for *L. monocytogenes* (MTCC 657) as 9 mm against roots and 8 mm against leaves and flowers, while stems not shown any inhibitory zone extracted in ethanol solvent. According to the inhibitory profile shown in this study, the tested bacteria were graded as – *S. aureus* (MTCC 96) >*S. typhi* (MTCC 734) >*S. paratyphi A* (MTCC 735) >*B. subtilis* (MTCC 554) >*E. coli* (MTCC119) >*P. vulgaris* (MTCC 426) >*K. pneumoniae* (MTCC 109) >*P. mirabilis* (MTCC 425) >*L. monocytogenes* (MTCC 657).

The results from antibacterial activity of the different extracts showed the potential of *Tridax procumbens* to inhibit the growth of wide variety of bacterial species. It has potential for anti-typhoidal & anti-paratyphoidal activity. Also *Tridax procumbens* has inhibitory potential against *E. coli*, *K. pneumoniae, P. vulgaris* and *B. subtilis.* The antibacterial potency of the *Tridax procumbens* plant studied by the researchers throughout the world, our results showed agreement with study of Aniel Kumar O and L. Mutyala Naidu (2008), Manjamalai et al., (2010), Tejaswini et al., (2011), Christudas et al., (2012) and Manjamalai et al., (2012). The data obtained from antibacterial activity of ethanolic extracts from the plant parts, the leaf showed activity against five bacterial species. Stem extract were active against three bacterial species, whereas flowers and roots were active against 1-2 bacterial species. Methanol extract from different plant parts showed optimum antibacterial activity, leaf extract active against eight bacterial species, stem extract against six bacterial species. Flower and root extracts both showed activity against nine bacterial species. As compared to methanol extract, acetone extract of different parts showed moderate activity, active against 4-8 bacterial species. Ethyl acetate and chloroform extracts showed mild antibacterial activity. Ethyl acetate extracts of leaf failed to show any activity, while stem showed activity against only one bacterial species, but flower & root extracts both showed activity against seven bacterial species. The chloroform extract of leaf failed to show any activity,

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the stem & flower both active for one bacterial species but root extracts active against six bacterial species. In ethyl acetate root extract, highest zone of 13 mm was recorded against *K. pneumoniae,* whereas in chloroform extract, highest zone of 12 mm was noted against both *S. paratyphi A* and *K. pneumoniae.*

According to the inhibitory potential shown by the different parts of the plant, parts can be graded as - Root > flower > leaf > stem. From the results of the present study, it is concluded that, the extracts had the antibacterial potential for the tested species; most susceptible bacteria included the *S. aureus*, *S. typhi*, *S. paratyphi A* and *B. subtilis,* whereas least susceptible bacteria was *L. monocytogenes.* Gram positive bacteria tested in the present study were found to be more susceptible to different extracts, than the Gram negative bacteria. Methanol root extract was found to be most active and ethyl acetate and chloroform extract of leaves and stems was found to be less active. The present study validates the medicinal use of this plant against the assayed pathogens.

**Table 1 -Phytochemical analysis of *Tridax procumbens* L. extracts.**

| Solvent system | Plant part / phytochemical | Carbohydrate | Cardiac Glycosides | Proteins | Flavonoids | Tannins | Terpenoids | Fixed oil & fats | Saponins | Alkaloids | Phenols | Resins | Phytosterols |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ethanol | Leaf | 1+ | 1+ | 2+ | 2+ | 1+ | 1+ | 1+ | - | 1+ | - | 1+ | 1+ |
| | Stem | 3+ | 1+ | 2+ | 2+ | 1+ | 1+ | 1+ | - | 1+ | 1+ | 1+ | 1+ |
| | Flower | - | 1+ | 2+ | 1+ | 1+ | 1+ | 1+ | - | 1+ | - | 2+ | 1+ |
| | Root | 1+ | 1+ | 2+ | 1+ | 1+ | 1+ | 1+ | - | 3+ | - | 2+ | 1+ |
| Methanol | Leaf | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ |
| | Stem | 1+ | 1+ | 2+ | 2+ | 1+ | 1+ | 1+ | 1+ | 1+ | 2+ | 1+ | 1+ |
| | Flower | 2+ | 2+ | 2+ | 3+ | 1+ | 1+ | 1+ | 1+ | 1+ | 2+ | 2+ | 1+ |
| | Root | 3+ | 2+ | 2+ | 2+ | 1+ | 1+ | 1+ | 1+ | 1+ | 2+ | 2+ | 1+ |
| Acetone | Leaf | 1+ | 2+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | - | 2+ | 1+ |
| | Stem | 2+ | 1+ | 2+ | 2+ | 2+ | 1+ | 1+ | 1+ | 1+ | - | 2+ | 1+ |
| | Flower | 2+ | 1+ | 1+ | 3+ | 1+ | 1+ | - | - | 1+ | - | 2+ | 1+ |
| | Root | 3+ | 1+ | 2+ | 2+ | 1+ | 1+ | - | - | 1+ | - | 1+ | 1+ |
| Ethyl Acetate | Leaf | 2+ | 1+ | 1+ | 1+ | 1+ | 1+ | - | - | 1+ | - | 1+ | 1+ |
| | Stem | 2+ | 1+ | 2+ | 1+ | 1+ | 1+ | 1+ | - | 2+ | - | 1+ | 1+ |
| | Flower | 2+ | 1+ | 2+ | 3+ | 1+ | 1+ | 1+ | - | 1+ | - | 1+ | 1+ |
| | Root | 2+ | 1+ | 2+ | 2+ | 1+ | 1+ | 1+ | 1+ | 2+ | - | 1+ | 1+ |
| Chloroform | Leaf | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | - | - | 1+ | - | 1+ | 1+ |
| | Stem | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | - | - | 1+ | - | 1+ | 1+ |
| | Flower | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | - | - | 1+ | - | 1+ | 1+ |
| | Root | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | - | 1+ | 1+ | - | 1+ | 1+ |

Abbreviations used: (-)Absent, (1+)Present in low concentration,
(2 +) Present in moderate concentration, (3+) Present in high concentration

**Table 2 Antibacterial activity of *Tridax procumbens* L. extracts.**

| Solvent system | Plant part / Bacterial species | B. subtilis | L. monocytogenes | P. vulgaris | E. coli | S. typhi | P. mirabilis | K. pneumoniae | S. aureus | S. paratyphi A |
|---|---|---|---|---|---|---|---|---|---|---|
| Ethanol | Leaf | 00 | 00 | 08 | 09 | 00 | 00 | 08 | 08 | 08 |
| | Stem | 00 | 00 | 00 | 08 | 00 | 00 | 08 | 08 | 00 |
| | Flower | 00 | 00 | 00 | 08 | 00 | 00 | 08 | 00 | 00 |
| | Root | 00 | 00 | 00 | 00 | 00 | 00 | 08 | 00 | 00 |
| Methanol | Leaf | 08 | 08 | 11 | 10 | 09 | 08 | 00 | 11 | 12 |
| | Stem | 08 | 00 | 00 | 09 | 09 | 08 | 00 | 09 | 11 |
| | Flower | 09 | 08 | 14 | 09 | 10 | 08 | 11 | 14 | 11 |
| | Root | 08 | 09 | 09 | 11 | 09 | 09 | 10 | 13 | 10 |

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| Solvent system | Plant part / Bacterial species | B. subtilis | L. monocytogenes | P. vulgaris | E. coli | S. typhi | P. mirabilis | K. pneumoniae | S. aureus | S. paratyphi A |
|---|---|---|---|---|---|---|---|---|---|---|
| Acetone | Leaf | 12 | 00 | 00 | 00 | 12 | 00 | 00 | 12 | 10 |
| | Stem | 14 | 00 | 00 | 08 | 11 | 00 | 12 | 13 | 13 |
| | Flower | 13 | 00 | 10 | 08 | 12 | 08 | 08 | 12 | 10 |
| | Root | 11 | 00 | 11 | 10 | 15 | 09 | 11 | 15 | 13 |
| Ethyl Acetate | Leaf | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| | Stem | 00 | 00 | 00 | 00 | 00 | 00 | 09 | 00 | 00 |
| | Flower | 09 | 00 | 11 | 09 | 08 | 00 | 11 | 10 | 11 |
| | Root | 10 | 00 | 12 | 09 | 09 | 00 | 13 | 10 | 11 |
| Chloroform | Leaf | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| | Stem | 00 | 00 | 00 | 00 | 00 | 00 | 09 | 00 | 00 |
| | Flower | 00 | 00 | 00 | 00 | 00 | 00 | 08 | 00 | 00 |
| | Root | 11 | 00 | 10 | 08 | 11 | 00 | 12 | 00 | 12 |

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