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# Review of antimicrobial spectrum of *Tridax procumbens* L. (Vishalyakarani) and estimation of flavonoids & phenols present in its leaves in ninety six well plate methods

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**Abstract**
Vishalyakarani or *Tridax procumbens* L is an Ayurvedic herb under asteraceae family with traditional use in wound healing purpose. It has many pharmacological properties including anti-microbial, anti-cancer, wound healing and anti-septic. The present study is a combined study consisting reviewing the anti-microbial spectrum of *Tridax procumbens* L. (Vishalyakarani) and quantification of flavonoids and phenols from its leaves especially from the Gandhamardhan hill, Odisha. This aims to equip researchers a useful data about the chemical contents of *T. procumbens*. The extract was prepared by using Soxhlet extractor and concentrated by distillation. Phytochemical analysis revealed the presence of flavonoids, phenols in extract and quantification by the high-throughput nine sixty well plate method. The review process yielded 748 research papers and after thorough discussion 81 papers were selected. The plant has many chemical compounds including flavonoids, essential oils, phenols and wide range of pharmacological activities from which we have included the anti-microbial activities. Historically and from Ramayana we have heard about the effectiveness of the Vishalyakarani in wound healing and in present review proves its worth. But there was a lack of quantification of two important phyto contents and it's done by high-throughput 96-well plate method.

**Keywords:** *Tridax procumbens*, hot oven method, soxhlet extractor, micro plate reader, 96-well plate method

## 1. Introduction
*Tridax procumbens* L. is a famous Ayurvedic herb under asteraceae family, also called as Coat Button and included in binomial nomenclature system by Linnaeus in 1753 [1]. It is seen as a dominant weed in many states such as Maharashtra, Odisha, Bihar [1]. It is the most potent species among 30 species of the genus Tridax [1]. This herb. *T. procumbens* have been used from ancient times to treat wounds, skin diseases and to stop blood clotting. It possesses anticoagulant, antileishmanial, antioxidants, anticancer, immune modulatory agent, and insecticidal, anthelmintic cardiovascular, antiseptic, antimicrobial, and insecticidal properties [1].

## 2. Methodology
### 2.1 Review Methodology
An internet based literature search of *T. procumbens* L was done using Google scholar, Science direct, Google patent, Pubmed, ACS and Mendley desktop databases of plants. Keywords used for literature survey, plant location, and antimicrobial spectrum of *T. procumbens* L. Undergraduate students of SSN Ayurveda College and Research Institute have participated in this exercise and study conducted in Pharmacognosy lab of SSN Ayurved College and RI, Paikmal, Bargarh (Odisha).The recommended reporting items for systematic reviews and meta-analysis (PRISM) guidelines were followed.

### 2.2 Pharmacological study's methodology
Different extracts were studied for their pharmacological potentials and here special emphasis was given for anti-microbial activities both *in vivo* and *in-vitro*.

### 2.3 Anti-microbial activities

The anti-bacterial activities with control, MIC/MBC by different methods are mentioned. Various extracts were used such as ether, chloroform, and ethanolic by Agar well diffusion method against *B. faecalis*, *B. subtilis*, *E. coli*, *P. aeruginosa*. The chloroform extracts was found to be effective against *B. faecalis* and *E. coli* [2]. After going through all these literatures the summery of the anti-microbial activities are reflected in the given table.

**Table 1:** Review summery of anti-microbial activities of *T. procumbens* L. *(*Vishalyakarani*)*

| Parts | Class of Compound | Methods | Effects | Standards | Doses | References |
|---|---|---|---|---|---|---|
| Leaves | Tannins, Steroids | Agar well diffusion | *B. subtilis* *B. faecalis* *E. Coli* | Ampicillin | 200µg 400µg 800µg. | Christudas (2012) [5] |
| Leaves Flowers | Flavonoids | ROS, Lipid peroxidation | *M. aeruginosa* | Ampicillin | 25mg/L-100mg/L for 10 days | Mecina (2019) [6] |
| Whole Plant Flowers | Tannins Carbohydrate | Disk diffusion methods | *E. Coli, S. typhi, S. paratyphi* | - | - | (Taddei –Rosas-Romero 2000) [7] |
| Fresh flowers | Essential oils | Tube dilution assay | *E. Coli, S. typhi S. paratyphi, M. segments* | - | 0.14-0.67mg/L | Joshi and Badakar 2012) [8] |
| Whole plant | Essential oils | Disk diffusion method | *S. aureus, E. Coli S. typhi, K. pneumonia Shigella flexneri* | Gentamycin | - | Mir. S and Dar. A 2016 [9] |
| Aerial part | Essential oils | Agar well Dilution method | *Microsporum fulvum, Microsporum gypsum,* | Griseofulvin | MIC 1.6 to 12.8 mg/mL | Policegudr *et al*.,2014 [10] |
| Fresh leaves | Essential oils | Disk diffusion | *S. aureus, S. pneumonia* | Norfloxacin-5µg/mL Cefepime-10µg/mL Gatifloxacin-15µg/mL | MIC/MFC 25-100µg/mL | Manjamlai *et al*.,2012a |
| Leaves | Essential oils | Agar well dilution | *Candida albicans Aspergillus, fumigates A. niger, Candida tropicalis* | - | MIF 50-500µg/mL | Manjamlai *et al*, 2012b. |
| Ariel Parts | Fatty acid | Disk diffusion method | *E. coli, P. mirabilis, S. aureus* | Streptomycin Ampicillin | 25-10 mg/mL | Andriana *et al*.,2019 [11] |
| Leaf | - | Disk diffusion method | *E. coli, S. aureus, S. pyogenes* | Ampicillin Cephalosporin | - | T.*et al*., 2018 |

### 2.4 Morphology

Tridax procumbens has a stem which can reach from to 8-30 inches (20-75 cm) long. The leaves are opposite, pinnate, oblong to ovate, and 1-2 inches (2.5-5 cm) long with cuneate bases, coarsely serrate margins, and acute apexes. The flowers have white rays and yellow disk flowers. They are about 0.4-0.6 inches (1-1.5 cm) wide, and held on a 4-12 inches (10-30 cm) long stalk. Flowering occurs in spring. Fruits are achenes that are dark brown to black in color, oblong, and 0.08 inches (2 mm) long, each with a head of pappus bristles that ovary from 0.12-0.24 inches (3-6 mm) long [1].

## 3. Phytochemical study

### 3.1 Flavonoids

The Phytochemical studies revealed that *Tridax procumbens* L. is a rich source of flavonoids, responsible for antioxidant, hepato protective, anticancer, antibacterial & wound healing properties. The flavonoids responsible for control the growth of toxin-producing bacteria in plants [3].

### 3.2 Total phenolic compounds estimation

*Tridax procumbens* L. leaves contents phenolic compounds having antioxidant properties. The anti-oxidants are preventing to damage cells by free radicals, the free radicals released during metabolic process of oxidation. The free radicals are reactive oxygen free radicals species (ROS), hydroxyl radicals (OH), superoxide anion radical ($O_2$), hydrogen peroxides ($H_2O_2$) and peroxyl (ROO). This free radical generates metabolites, which are damage cell membrane DNA. The free radicals are causing carcinogenesis [4].

#### 3.2.1 Estimation of Phenols and Flavonoids by 96 well plate method


**Fig 1:** Micro Plate Reader

**3.2.2 Estimation of total phenols**

**3.3 Reagents for total phenol content:**

1. **Folin reagent 10% Solution:** In the measuring jar Folin reagent is taken 10 ml and adjust 100 ml of volume with the distilled water
2. **Sodium Carbonate 5% solution:** In the volumetric flask 5g of Sodium Carbonate is taken and make up with distilled water up to mark in 100 ml.

**3.4 Procedure:** 2 mg of aqueous extract is taken in vial. 1.5 ml of folin reagent is added, 1.5 ml of sodium carbonate solution and 2 ml distilled water is added and made a solution for 96 well plate method solution kept in 37 °C. From this solution 0.1, 0.2, 0.3 ml is added in three wells. The absorbance of the reaction mixture was read at 765 nm using a visible-UV micro plate kinetic reader [4].

**3.5 Estimation of total flavonoids**

**3.5.1 Reagents for flavonoids (Total Flavonoids Content)**

1. **Aluminum Chloride 10% solution:** In the volumetric flask 10 g of aluminum chloride is taken and make up with distilled water up to mark in 100 ml.
2. **Sodium Hydroxide 4% solution:** In the volumetric flask 4 g of sodium hydroxide is taken and make up with distilled water up to mark in 100 ml.

**3.5.2 Sample:** 2 mg of aqueous extract is taken in vial. 1.5 ml of aluminum chloride is added, 1.5 ml of sodium hydroxide solution and 2 ml distilled water is added and made a solution for 96 well plate method solution kept in 37 °C. From this solution 0.1, 0.2, 0.3 ml is added in three wells. The absorbance of the reaction mixture was read at from 367 to 510 nm using a visible-UV micro plate kinetic reader [4].

**4. Result**

**4.1 Estimation of Phenols and Flavonoids by 96 well plate method**

**TFC X= Readings obtained 0.420, 0.426, 0.419**

Y=0.0098

Amount of TFC of 200 µL contains = $\frac{0.420}{Y}$ = 42.85µg

For 1000 µL contains = $\frac{1000}{200} \times 42.85$ =214.25 (For 2 mg of extract)

For 3.583 gm extract = $\frac{3.583 \times 1000 \times 214.25}{2 \times 1000}$ = 383.82 mg

**TPC X= Readings obtained 0.389, 0.380, 0.386**

Y=0.0166

Amount of TFC of 200 µL contains = $\frac{0.384}{0.0166}$ = 23.43 µg

For 1000 µL contains= $\frac{1000}{200} \times 23.43$ =117.15 (For 2 mg of extract)

For 3.583 gm extract = $\frac{3.583 \times 1000 \times 117.15}{2 \times 1000}$ = 209.87 mg

The micro plate reader method yielded the following results

**4.2 Total Phenol Content:** 209.87 mg= 5.85%

**4.3 Total Flavonoids Content:** 383.82 mg=10.71%

**5 Conclusions**

Review of different journals leads to the conclusion that its leaves are definitely anti-microbial in different concentrations for a varieties of microorganisms. Leaves collected from the Gandhamardhan hill forest contain 10.71% flavonoids & 5.85% phenolic compound in aqueous crude which seems marvelous and opens new avenues for further isolation and quantification of other vital phyto constituents.

**6. Future study:** Preclinical studies could be done to test its effectiveness in animal models and latter on clinical trials could be planned.

**7. Conflict of Interest:** Nil

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