

# Pharmacological activities and effective in-vitro propagation of *Tridax procumbens*: An ethnomedicinal powerhouse plant

Kirti Ahlawat<sup>1</sup>, Hitendra Kaur Sidhwani <sup>2</sup>, Shalini Sharma<sup>2</sup>, Aditi Arya<sup>1\*</sup>, Nitesh Singh<sup>3</sup>, Sapna Grewal<sup>4</sup>, Sonia Goel<sup>3\*</sup>

<sup>1</sup>Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, 131039, Haryana, India.

<sup>2</sup>Department of Chemistry, Medicaps University, Indore-453331, Madhya Pradesh, India.

<sup>3</sup>Faculty of Agricultural Sciences, SGT University, Gurugram, 122505, Haryana, India.

<sup>4</sup>Guru Jambheshwar University of Science and Technology, Hisar, 125001, Haryana, India.

#1<sup>st</sup> Author – Kirti Ahlawat and Aditi Arya – Both Authors Contributed Equally

\*Corresponding Author – Dr. Sonia Goel ([soniagoelbiotech@gmail.com](mailto:soniagoelbiotech@gmail.com))

Dr. Aditi Arya ( [aditi29parkash@gmail.com](mailto:aditi29parkash@gmail.com) )

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## KEYWORDS

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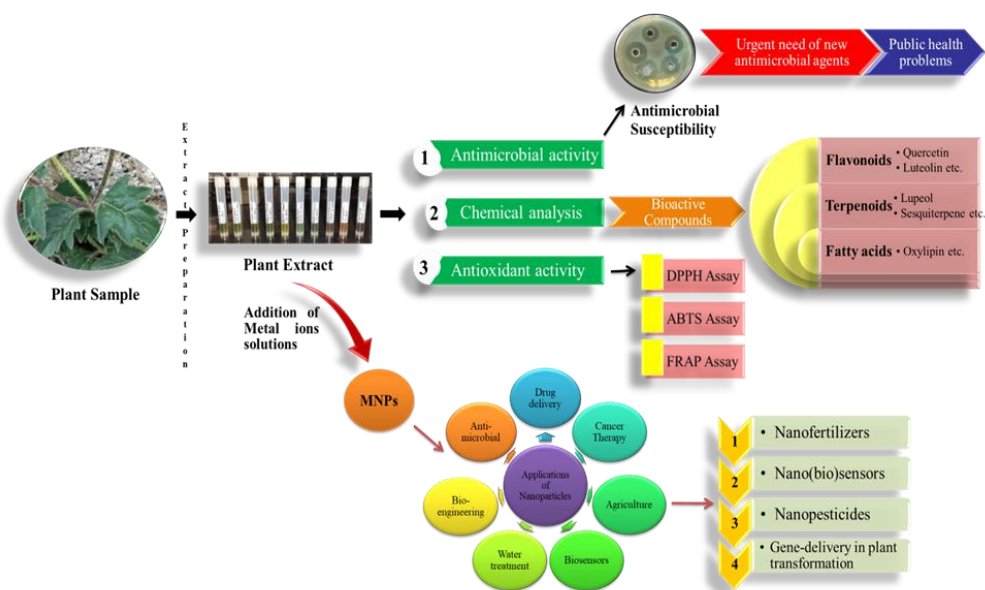
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## Abstract

*Tridax procumbens* is an invasive yet pharmacologically valuable weed widely distributed across India. This review consolidates current knowledge on its botanical traits, phytochemical diversity, toxicological aspects, and pharmacological potential, with emphasis on its ethnobotanical relevance among tribal communities such as the Paliyar and Kuruma. The study highlights recent evidence supporting its antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and wound-healing properties, linked to its diverse secondary metabolites. Notably, advances in tissue culture techniques demonstrate the potential of optimizing plant growth regulators to enhance biomass and metabolite yield, providing a sustainable strategy for large-scale bioactive compound production. Despite its wide traditional use, systematic studies delineating specific bioactive molecules and their mechanisms remain limited, underscoring the need for targeted molecular and clinical investigations to fully harness the therapeutic promise of *T. procumbens*.



**Graphical Abstract: The application of *Tridax procumbens* extract made from whole plant as bioactive compounds and metallic nano-particles**

## Introduction

Medicinal plants serve as vital raw materials for the pharmaceutical industry, contributing to the development of diverse therapeutic formulations. Among these, *Tridax procumbens* holds significant ethnomedicinal importance across tropical and subtropical regions. Traditionally, it has been used to treat wounds, fever, bronchial infections, liver ailments, and inflammation in regions such as India, Africa, and Central America (Aarefoddin et al., 2025). In African traditional medicine, it functions as an antibacterial and antiparasitic remedy for humans and animals, while in Ayurveda, it is valued for its hepatoprotective and wound-healing effects. Similarly, in Latin America, particularly Guatemala, *T. procumbens* is utilized for its anti-

inflammatory, antimalarial, and antidiabetic properties. The World Health Organization (WHO) reports that over 21,000 plant species are used globally in traditional medicine (Babu et al., 2020; Singh et al., 2021b; Thakur et al., 2022), many of which support the herbal and aromatic industries in India (Srivastava et al., 2023; Dixit et al., 2023). Belonging to the family Asteraceae and commonly known as Tridax daisy or coat button, *T. procumbens* grows abundantly in cultivated fields, wastelands, and along roadsides throughout tropical and subtropical regions. Its prolonged seed germination period and ecological adaptability contribute to its persistence and widespread distribution.

Ecologically, *T. procumbens* demonstrates high adaptability, as reflected in studies from the Kanyakumari district of India, where it recorded the highest Importance Value Index in red soils, indicating dominance across diverse ecosystems. Plant growth traits such as height, leaf area, and biomass decrease under shaded conditions. The plant's calcium accumulation varies with soil composition, revealing distinct ecotypes in areas with calcareous parent materials (Finch-Savage & Bassel, 2016). Phytochemical analyses have revealed the presence of alkaloids, steroids, carotenoids, flavonoids, fatty acids, phytosterols, tannins, and minerals (Timalsina et al., 2021). Medicinally, it has been used to manage diarrhea, malaria, stomach ache, dysentery, and hair loss (Jude et al., 2009). The presence of oleanolic acid contributes to its antidiabetic activity, and more than 21 traditional uses have been reported for the species.

Recent advancements in plant biotechnology have enabled efficient propagation and conservation of *T. procumbens*. In vitro micropropagation techniques support large-scale production of genetically stable plantlets, independent of seasonal variations, while ensuring conservation of valuable germplasm (Saini et al., 2008; Singh et al., 2021a). Biotechnological methods such as callus culture, synthetic seed development, and cryopreservation enhance both ex situ

conservation and secondary metabolite production (Nalawade et al., 2003). The regulation of growth and metabolite synthesis is primarily influenced by plant growth regulators, particularly auxins and cytokinins, which control tissue differentiation and organogenesis (Sheoran et al., 2021).

Globally, *T. procumbens* represents a model species linking traditional medicine with modern pharmacological and biotechnological research. Previous studies have explored its ethnomedicinal uses, phytochemistry, and pharmacology in isolation. The present review integrates these domains to provide a holistic understanding of its therapeutic potential and conservation strategies. By combining ethnobotanical knowledge with in vitro propagation and phytochemical profiling, this work establishes a multidisciplinary framework for the sustainable utilization and scientific validation of *T. procumbens*, thereby advancing its potential for standardized, large-scale medicinal applications.

## Methodology

In the present review, a systematic literature-based synthesis was performed to compile and evaluate existing data on *Tridax procumbens*, focusing on its phytochemical diversity, ethnopharmacological relevance, and in vitro propagation studies. No new laboratory experiments were conducted; all information presented herein was derived from

previously published research. A comprehensive literature search was conducted across electronic databases including PubMed, Scopus, ScienceDirect, and Google Scholar covering the period 2000–2025. The predefined search terms included combinations of the following keywords: “*Tridax procumbens*,” “phytochemical composition,” “pharmacological activity,” “tissue culture,” “micropropagation,” “secondary metabolites,” and “ethnomedicine.” Boolean operators (“AND,” “OR”) were used to refine search results.

Article screening and selection followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency and reproducibility. The Cochrane Risk of Bias 2.0 (RoB 2.0) tool was used to evaluate the quality and reliability of included experimental studies. Data extraction was carried out independently, focusing

on study objectives, plant part investigated, extraction techniques, identified phytochemicals, bioassay types, and culture conditions. For studies describing in vitro propagation, details such as the type and concentration of plant growth regulators (auxins, cytokinins), culture media composition, incubation conditions, and explant type were recorded. Reports specifying replicate numbers and statistical methods such as ANOVA used to optimize culture conditions were highlighted to ensure methodological reproducibility. All extracted data were synthesized qualitatively and presented in comparative tables summarizing phytochemical profiles, pharmacological properties, and tissue culture outcomes. Additionally, a methodological (table 1) was designed to illustrate the sequential approach adopted in this review from database search and article screening to data synthesis and interpretation for enhanced clarity and reference.

Table 1: Methodological Framework for the Review of *Tridax procumbens*

| Step                      | Process / Description                       | Details / Tools Used                                                                              |
|---------------------------|---------------------------------------------|---------------------------------------------------------------------------------------------------|
| <b>1. Database Search</b> | Comprehensive search across major databases | PubMed, Scopus, ScienceDirect, Google Scholar; search period 2000–2025                            |
| <b>Keywords Used</b>      |                                             | “ <i>Tridax procumbens</i> ”, “phytochemistry”, “pharmacology”, “ethnomedicine”, “tissue culture” |
| <b>2. Screening</b>       | Title and abstract screening                | Excluded unrelated, duplicate, and non-English studies                                            |

| Step                                   | Process / Description   | Details / Tools Used                                                                                                                                                            |
|----------------------------------------|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>3. Eligibility Assessment</b>       | Full-text evaluation    | Included peer-reviewed studies focusing on phytochemical composition, pharmacological relevance, ethnobotanical significance, and in-vitro propagation or metabolite production |
| <b>4. Study Selection</b>              | PRISMA-guided synthesis | Final studies selected after systematic screening                                                                                                                               |
| <b>5. Risk of Bias Assessment</b>      | Quality evaluation      | Cochrane Risk of Bias 2.0 (RoB 2.0) tool                                                                                                                                        |
| <b>6. Data Extraction and Analysis</b> | Categorization of data  | (1) Ethnobotanical and pharmacological data; (2) Phytochemical characterization; (3) In-vitro culture optimization and metabolite synthesis                                     |

## Results and Discussion

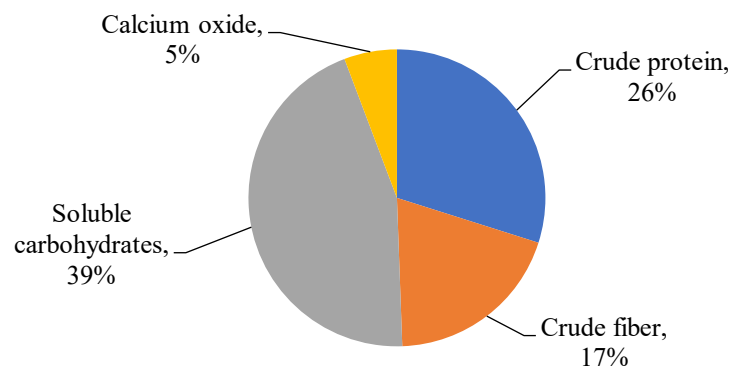
### Morphological Structure and Chemical Composition

*Tridax procumbens* leaves are simple, ovate and stipulated. Flowers have capitulum inflorescence and basal placentation in this plant. *Tridax* produces many rough achenes. Seeds are germinated at high temperature and sensitive to salt concentration and water stress (Chauhan and Johnson, 2008). There are different bioactive compounds present in *T. procumbens* such as oleanolic acid, fumaric acid, tannin, and fl-sitosterol. The leaves extract contains bioactive compounds such as flavonoids (flavones and catechins), alkaloids, tannins, carotenoids and saponins (Christudas *et al.*, 2012; Rajak *et al.*, 2023). Some of the secondary metabolites like luteolin, quercetin, glucoluteolin, and isoquercetin are available in flowers. The plant contains high

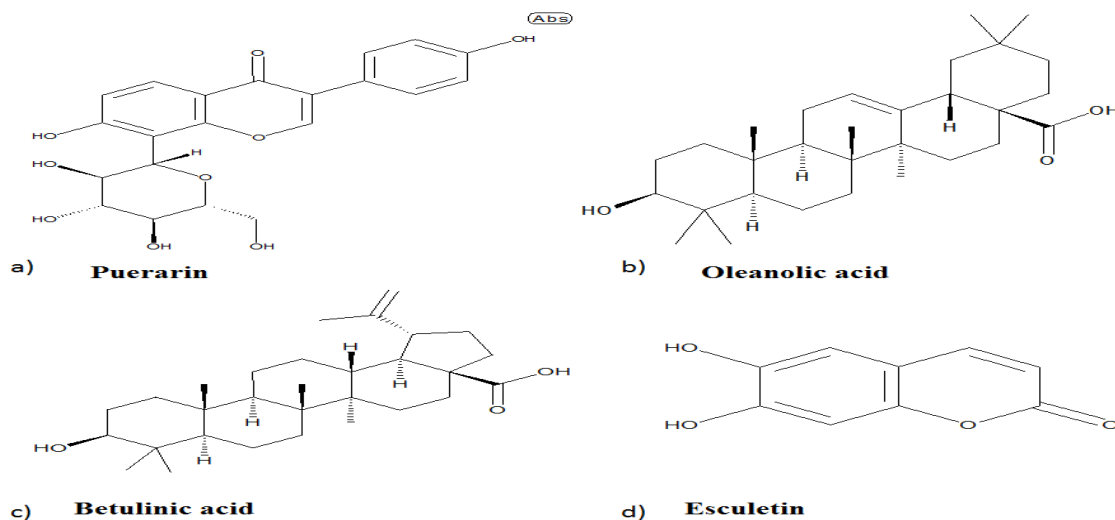
moisture content of 90.05% in leaf and 88.30% in stem and around 39% soluble carbohydrates, 26% crude protein, 17% crude fiber were measured in it as mentioned in Figure 1 and about 5% calcium oxide is present in the leaves of this plant (Christudas *et al.*, 2012). In addition to that, the plant contains abundant minerals, including sodium, zinc, iron, copper, and manganese, as well as trace elements such as magnesium, potassium, phosphorus, calcium, and selenium (Jude *et al.*, 2009; Ingle, *et al.*, 2014; Sahariah *et al.*, 2023). Many isolated constituents from *T. procumbens*, including saturated and unsaturated fatty acids, lipids, and flavonoids, terpenoids, polysaccharides, like puerarine,  $\beta$ -sitosterol, dexamethasone, esculetin, fumaric acid, oleanolic

acid, betulinic acid, quercetin, isoquercetin, lupeol, centaureidin, and luteoline were discovered in various reports from pharmaceutical chemistry. From the ethyl acetate extract of *T. procumbens* roots, two different compounds were isolated named  $\beta$ -sitosterol-3-O- $\beta$ -D-glucopyranoside (daucosterol) and 3',5-dihydroxy-4',3,6-trimethoxyflavon-7-O- $\beta$ -glucopyranoside (centaurein) (Do Nguyen *et al.*, 2015). Elucidation

of two new flavones structures was also reported in *Tridax procumbens* based on their spectral methods and their chemical analysis named 6,8,3'-trihydroxy-3,7,4'-trimethoxyflavone and 8,3'-dihydroxy-3,7,4'-trimethoxy-6-O- $\beta$ -D-glucopyranosyl flavone. Structural representation of some biochemical compounds is shown below in figure 2.



**Figure 1. Percentage of various metabolites extracted from the *T. procumbens***



**Figure 2. Chemical structure of important secondary metabolites extracted from *T. procumbens* a) Puerarin, b) Oleanolic acid, c) Betulinic acid, d) Esculetin**

### ***In vitro* establishment and multiplication**

Different combinations of Murashige and Skoog medium with growth regulators were taken in many research for the induction of *in-vitro* callus and flowering in *T. procumbens*. In a study, a solitary shoot development per node was seen on MS medium treated with BAP concentration. It was noticed that the explants grown on Murashige and Skoog medium with a low concentration of BAP show a high number of multiple shoot bud formation. Whereas high concentration of BAP shows an inhibitory effect on multiple shoot induction in *T. procumbens*. The shoot tip and nodal segment explants were cultivated in MS media with various concentrations of BAP and KIN (0.5, 1.0, 2.0, 3.0, and 4.0). The maximum culture response (90%) was seen in 1.0 mg/l BAP, whereas the lowest (35%) was seen in 4.0 mg/l KIN. All these responses were observed after 45 days of culturing. Other plant growth regulators, alone or in combination, were unable to stimulate shoot proliferation in *T. procumbens* nodal explants (Wani *et al.*, 2010).

### **Callus Induction**

The use of callus culture can aid in the generation and isolation of alkaloids. Plant growth regulators like auxin and cytokinin are the most important growth regulative for influencing development and

proliferation in plant tissue and organ culture. Secondary metabolites can often be found in large quantities in tissue growing as callus masses. The accumulation of biomass and secondary metabolites can be increased by altering the plant growth regulator. The auxin 2, 4 D, in conjunction with BAP or Kinetin, was found to be the greatest source of auxin for callus induction in *Tridax* explants, followed by auxin NAA in combination with BAP (Wani *et al.*, 2010). *In vitro* callus formation can promote the mass production of bioactive chemicals with health advantages from the *Tridax procumbens* plant.

### ***In vitro* flowering**

Flowering is a complicated process that is influenced by both internal and external influences, and it is extremely rare to induce flowering *in vitro*. After 3–4 weeks of cultivation on Murashige and Skoog medium supplemented with different absorptions of BAP and adenine sulphate, *in vitro* flowering began (Kulus & Tymoszek, 2024). BAP and adenine sulfate with a concentration of 1.0mg/L and 100mg/L respectively could only trigger the floral development *in vitro* while the other concentrations couldn't. BAP has been discovered to be a key element in the production of floral



organs in regenerated plantlets, as well as a growth regulator. *T. procumbens* can be successfully multiplied in large quantities using the described *in vitro* procedure.

The in-vitro propagation studies on *T. procumbens* as shown in Table 2 reveals a high efficiency of shoot and root induction. The development of reproducible micropropagation protocol serves as a critical platform for both the commercial

utilization and conservation of *T. procumbens* germplasm. Biotechnologically, this system facilitates large scale, continuous production of genetically uniform plantlets and supports the regulated biosynthesis of pharmacologically significant metabolites, including flavonoids and sterols, under optimized in vitro culture conditions (Preetpal *et al.*, 2017).

**Table 2: Effect of different concentrations of Cytokinins and Auxins on *in vitro* propagation of *T. procumbens***

| S. No. | Plant Part Used   | Media Used | Treatment                                   | Effect                                                | References                       |
|--------|-------------------|------------|---------------------------------------------|-------------------------------------------------------|----------------------------------|
| 1      | Shoot tip explant | MS Media   | BAP at 1.0 mg/l                             | Show a high frequency of multiple shoot bud formation | (Ushahra and Malik, 2013)        |
| 2      | Explant           | MS Media   | 2, 4 D + BAP/ Kinetin                       | Callus induction                                      | (Wani <i>et al.</i> , 2010)      |
|        |                   |            | NAA + BAP                                   | Callus induction                                      | (Wani <i>et al.</i> , 2010)      |
| 3      | Axial Parts       | MS Media   | BAP+ adenine sulphate (1.0mg/L and 100mg/L) | Induce <i>in vitro</i> flowering                      | (Kulus & Tymoszuk, 2024)         |
|        |                   | MS Media   | IBA (1.0 mg/l)                              | Root induction                                        | (Jesmin <i>et al.</i> , 2013)    |
| 4      | Nodal Segment     | MS Media   | BAP + NAA(2.0+0.5 mg/L)                     | Moderate Callus Formation                             | (Jesmin <i>et al.</i> , 2013)    |
| 5      | Nodal explants    | MS Media   | BAP (0.25mg/L)                              | Shoot multiplication                                  | (Rodrigues <i>et al.</i> , 2023) |
| 6      | Nodal explants    | MS Media   | IAA+BAP (0.6+1mg/L)                         | Highest shoot height                                  | (Rodrigues <i>et al.</i> , 2023) |
| 7      | Auxillary bud     | MS Media   | BA+NAA (5+0.5 mg/L)                         | Shoot induction and multiplication                    | (Mabrouk <i>et al.</i> , 2021)   |
| 8      | Auxillary bud     | MS Media   | BA+NAA (1.5+0.0625 mg/L)                    | Shoot induction and multiplication                    | (Ferreira <i>et al.</i> , 2021)  |
| 9      | Auxillary bud     | MS Media   | BA+NAA (1.5+0.0625 mg/L)                    | Shoot elongation                                      | (Ferreira <i>et al.</i> , 2021)  |
| 10     | Auxillary bud     | MS Media   | BA+IAA (0.5+0.1 mg/L)                       | Shoot induction and multiplication                    | (de Souza <i>et al.</i> , 2019)  |



|    |                |                         |                                                             |                              |                                   |
|----|----------------|-------------------------|-------------------------------------------------------------|------------------------------|-----------------------------------|
| 11 | Auxillary bud  | MS Media                | mT + IAA (0.26+0.1mg/L)                                     | Shoot elongation             | (de Souza <i>et al.</i> , 2019)   |
| 12 | Auxillary bud  | MS Media                | BA+IAA (0.5+0.1 mg/L)                                       | Shoot rooting                | (de Souza <i>et al.</i> , 2019)   |
| 13 | Nodal explants | MS Media                | BAP (1.0mg/L)                                               | Multiple shoot proliferation | (Kulus & Tymoszek, 2024)          |
| 14 | Nodal explants | Half strength MS Medium | IBA (0.5mg/L)                                               | High frequency rhizogenesis  | (Kulus & Tymoszek, 2024)          |
| 15 | Nodal explants | MS Media                | BAP + Adenine sulfate (1.0 + 100mg/L)                       | <i>In vitro</i> flowering    | (Kulus & Tymoszek, 2024)          |
| 16 | Auxillary bud  | MS Media                | BA (0.7 mg/L)                                               | Shoot induction              | (Thaniarasu <i>et al.</i> , 2015) |
| 17 | Auxillary bud  | MS Media                | BA+TDZ+Adenine sulfate (0.7+1.0+25 to 100mg/L)              | Shoot multiplication         | (Thaniarasu <i>et al.</i> , 2015) |
| 18 | Auxillary bud  | MS Media                | BA+TDZ+Adenine sulfate+GA <sub>3</sub> (0.7+1.0+50+0.5mg/L) | Maximum shoot length         | (Thaniarasu <i>et al.</i> , 2015) |

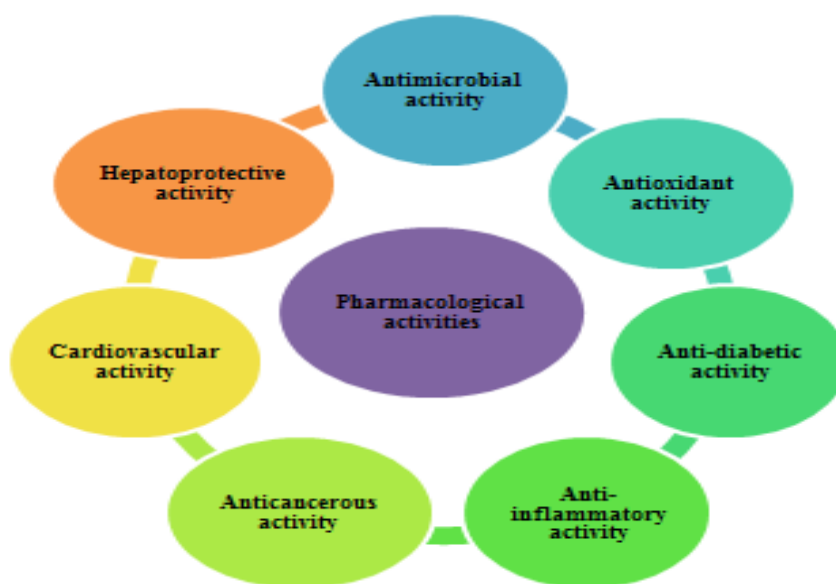
### Ethnomedicinal uses and Pharmacological and Therapeutic Properties

Verbally transmitted knowledge of traditional medicine of ethnic communities and their practices are scientifically known as “ethnomedicine” which helps in the study of their traditional way of treatment (Chattopadhyay, 2009; Chattopadhyay *et al.*, 2010; Singh *et al.*, 2022; Sahu *et al.*, 2023; Rajak *et al.*, 2025). In India, our native and today's generation mostly uses plants in the treatment of many diseases and this term is known as "ethnobotanical medicine" (Fabricant and Farnsworth, 2001; Parshad *et al.*, 2024) (Figure 3). The traditional medical practices distributed with the cultural perception of health, diseases, and illnesses were widely attributed by the ethnomedicines that address different healing

proceedings and healthcare processes. Most individuals are turning to medicinal herbs in search of treatments and health measures. Synthetic chemicals have a lot of negative side effects; however natural remedies are free from those side effects. As a result, researchers are paying close attention to the use of eco-friendly and bio-friendly plant-based goods these days. “Ayurveda” is the most known traditional medicine system in India which prevails even during the Indus Valley Civilization era. "Ayurveda" emphasizes the importance of maintaining a healthy balance between the human body, mind, and environment. Ethnic communities or ethnomedicine used different medicinal resources and treatments based

on plants by utilizing various plant components, such as fruits, leaves, roots, bark, or even seeds. *T. procumbens* have antimicrobial, antibiotic efficacy, antioxidant, insecticidal, anti-inflammatory activity, wound healing activity, dysentery, and diarrhoea. It's mostly used in India as a wound healer, antifungal, anticoagulant, and insect repellent. The leaf extract is used to stop bleeding, in wound healing, hair tonic, and cure skin infections. For eradication of Chromium or [Cr (VI)] from industrial wastewater, it is used as an absorbent. It is a prominent medication for liver disorders or aside from gastritis and heartburn, it has a hepatoprotective nature. A significant increase in the ethanol and water extracts of *T. procumbens* was described by (Talekar *et al.*, 2012) in the wound tensile strength and hydroxyproline, and also in the content of hexosamine and collagen when correlated to the

control group (Talekar *et al.*, 2012; Agyare *et al.*, 2016). Each part of this plant is useful for ethnomedicines and various pharmacological activities. A good amount of oleanolic acid was obtained from the *Tridax*, when it was tested against the glucosidase, it was found to be a probable anti-diabetic agent. The ethnomedicinal uses reported in this plant represented in table 3. Based on the medicinal herbs Indian researchers developed a filter system that is used for the easy and quick removal of 'fluorine' present in drinking water. *T. procumbens* were previously studied for their ability to remove toxic heavy metals from water. This plant has recently been discovered to play an important role in defluoridation, where it serves as a cost-effective way to defluoridate water in areas where natural fluorine levels in groundwater are high, such as India (Chauhan *et al.*, 2024).



**Figure 3: Pharmacological activities of *Tridax procumbens***

**Table 3: Ethnomedicinal uses of *Tridax procumbens***

| S. No. | Plant Parts | Mode of preparation       | Treatment                                          | Reference                                             |
|--------|-------------|---------------------------|----------------------------------------------------|-------------------------------------------------------|
| 1      | Leaves      | Leaf paste                | Wound healing                                      | (Rangaswamy and Vanitha, 2017)                        |
| 2      | Leaves      | Decoction                 | Against Dysentery, Diarrhoea                       | (Saxena and Albert, 2005)                             |
| 3      | Leaves      | Aqueous extract           | Prevent hair falling and promote hair growth       | (Saxena and Albert, 2005; Rathi <i>et al.</i> , 2008) |
| 4      | Leaves      | Leaf decoction            | Prevent haemorrhage from cuts, wounds, and bruises | (Saxena and Albert, 2005)                             |
| 5      | Leaves      | Aqueous extract           | Insect repellent activity                          | (Rajkumar <i>et al.</i> , 2007)                       |
| 6      | Leaves      | Leaf powder + 100ml water | Bovine Mastitis in udder tissue of cattles         | (Dhanabalan <i>et al.</i> , 2008)                     |
| 7      | Leaves      | Leaf Extract              | Against conjunctivitis                             | (Nia <i>et al.</i> , 2003)                            |
| 8      | Leaves      | Methanolic extract        | Against human lung cancer cells                    | (Syed <i>et al.</i> , 2020)                           |
| 9      | Leaves      | Ethanolic extract         | Burn wound model, Excision wound model             | (Shrivastav <i>et al.</i> , 2020)                     |
| 10     | Leaves      | Ethanolic extract         | Anti-inflammatory Activity                         | (Berlin Grace <i>et al.</i> , 2020)                   |
| 11     | Whole plant | Waterleaf decoction       | Wound healing                                      | (Talekar <i>et al.</i> , 2012)                        |

|    |                                      |                                                                                     |                           |                                |
|----|--------------------------------------|-------------------------------------------------------------------------------------|---------------------------|--------------------------------|
| 12 | Whole plant                          | Dried powdered of the whole plant and methanol was used                             | Against Chiclero's Ulcer  | (Croft and Coombs, 2003)       |
| 13 | Aerial parts (leaves, flowers, stem) | Powdered extract macerated with 50% methanol, petroleum ether, and water separately | Against Diabetes Mellitus | (Pardeshi and Bhiungade, 2016) |
| 14 | Aerial parts (leaves, flowers, stem) | Aerial parts were used with petroleum ether                                         | Cure Liver disorders      | (Hemalatha, 2008)              |
| 15 | Flower                               | Methanolic extract                                                                  | Prevent Hemostasis        | (Kedari, A., & Gupta, 2017)    |

**Antibacterial Activity:** Due to the global expansion of new viral, fungal and bacterial illness this plant shows a positive influence to flourish safely and novel antibiotics in contemporary medicines. There were several reports which influence the use of this plant as traditional medicines to cure different pathogenic diseases (Ingole *et al.*, 2022; Singh *et al.*, 2023a; Singh *et al.*, 2023b; Kumar *et al.*, 2023; Kumar *et al.*, 2024). It was recently revealed that the whole parts of *T. procumbens* have antimicrobial activity on various species of bacteria. The plant's antibacterial properties were tested against the *Pseudomonas aeruginosa*. The secretions like tracheal secretions and bronchoalveolar lavage from the ventilator-associated with pneumonia patient were used for the isolation of *Pseudomonas* a nosocomial strain. Best antibacterial activity was reported against *Pseudomonas aeruginosa* by the

ethanolic extract. At a concentration of 5 mg/ml, the zone of inhibition was enlarged. Comparing this strain with some control medication like ciprofloxacin, cephotaxime, augmentin, and even ticarcillin showed resistance whereas reactive only to imipenem. The disc diffusion assay revealed that the whole plant extract had antimicrobial activity. Two gram-positive (*Staphylococcus*, *Bacillus subtilis*) and two gram-negative (*Pseudomonas aeruginosa* and *E. coli*) bacteria were employed in this whole plant extract. From this report, it was observed that the whole plant extract showed effective antibacterial activity only against the *Pseudomonas aeruginosa*. According to the study, Gram-negative bacteria exhibited a larger zone of inhibition (Dhasarathan *et al.*, 2011). The antibacterial activity of *T. procumbens* leaf extract tested against *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholera*, and *Proteus mirabilis* using various solvents such as

methanol, chloroform, petroleum ether, and hexane. The Agar well diffusion method had been used to assess antibacterial activity. The study's findings suggest that the methanol extract contains a greater number of bioactive compounds compared to the hexane extract (Sathya Bama *et al.*, 2012). The antibacterial activity of the methanol extract was most effective against *Shigella flexneri* and *Salmonella typhi*, but least effective against *E. coli* (Muthusamy, *et al.*, 2013). Different phytochemicals such as tannins, flavonoids, ethyl esters, sterols, saponins, phenols, and unsaturated fatty acids are accountable for the antimicrobial activity observed. Table 4 provided below demonstrates the varying antibacterial

activities of different extracts, which elucidates why the plant has been utilized in traditional folk medicine to alleviate a range of bacterial infections such as diarrhoea, dysentery, and gastrointestinal disorders. Antibacterial activity was shown by different types of biocompounds like flavonoids, alkaloids, tannins, hydroxycinnamates, phytosterols, and many more. All these biocompounds contain different types of chemicals out of which luteolin, kaempferol, apigenin, catechins, myricetin, akuammidine are the most common biochemicals present in the *Tridax procumbens* leaves which show antibacterial or antimicrobial activity.

**Table 4: Bioactive compounds and their pharmacological activities**

| S. No. | Chemical Compound                                  | Plant Part                  | Chemical Type | Pharmacological activity                                           | Reference                            |
|--------|----------------------------------------------------|-----------------------------|---------------|--------------------------------------------------------------------|--------------------------------------|
| 1      | Ethyl ether                                        | Whole Plant                 | Flavonoid     | Antifungal activity                                                | (Jindal, A., <i>et al.</i> , 2012)   |
| 2      | Lupeol                                             | Whole Plant                 | Terpenoid     | Anticancerous activity                                             | (Mahato and Chaudhary, 2005)         |
| 3      | Procumbenetin                                      | Leaves, flower, stem        | Flavonoid     | Hepatoprotective activity                                          | (Ravikumar <i>et al.</i> , 2005)     |
| 4      | Petroleum ether                                    | Leaves                      | Flavonoid     | Antibacterial activity                                             | (Amutha <i>et al.</i> , 2019)        |
| 5      | $\beta$ -sitosterol-3-O- $\beta$ -D-Xylopyranoside | Flower                      | Steroid       | Antiseptic activity, Insecticidal activity, Parasitocidal activity | (Saxena and Albert, 2005)            |
| 6      | Sesquiterpene Lactone                              | Leaves, flower, stem, roots | Terpenoid     | Immunomodulatory activity                                          | (Gubbiveeranna <i>et al.</i> , 2016) |
| 8      | Ciprofloxacin, Cephataxime                         | Whole Plant                 | Quinolone     | Antibacterial activity                                             | (Amutha <i>et al.</i> , 2019)        |

|    |                                                                         |                      |                               |                                                                                                                                         |                                                                   |
|----|-------------------------------------------------------------------------|----------------------|-------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|
| 9  | Chloroquine                                                             | Leaves               | Aminoquinoline                | Hepatoprotective activity                                                                                                               | (Ravikumar <i>et al.</i> , 2005)                                  |
| 10 | Atropine                                                                | Leaves               | Tropane                       | Hypotensive effect                                                                                                                      | (Salahdeen <i>et al.</i> , 2004)                                  |
| 11 | Lectine                                                                 | Leaves               | Carbohydrate binding proteins | Immunomodulatory activity                                                                                                               | (Oladunmoye <i>et al.</i> , 2006)                                 |
| 12 | Lipopolysaccharide, D-galactosamine                                     | Leaves               | Lipoglycans                   | Hepatoprotective activity                                                                                                               | (Ravikumar <i>et al.</i> , 2005)                                  |
| 13 | Chloroform                                                              | Leaves               | Trichloromethane              | Antibacterial activity                                                                                                                  | (Do Nguyen <i>et al.</i> , 2015)                                  |
| 14 | 8,3'-dihydroxy-3,7,4'-trimethoxy-6-O- $\beta$ -D-glucopyranosyl flavone | Leaves, flower, stem | Flavone                       | Antibacterial activity                                                                                                                  | (Xu <i>et al.</i> , 2010)                                         |
| 15 | 6,8,3'-trihydroxy-3,7,4'-trimethoxyflavone                              | Leaves, flower, stem | Flavone                       | Antibacterial activity                                                                                                                  | (Xu <i>et al.</i> , 2010)                                         |
| 16 | Oxylipin                                                                | Whole Plant          | Fatty acids                   | Antipromastigote Activity                                                                                                               | (Martin-Quintal <i>et al.</i> , 2010)                             |
| 17 | (3S)-16,17-didehydrofalcarninol                                         | Whole Plant          | Oxylipin                      | Leishmanicidal effect                                                                                                                   | (Martin-Quintal <i>et al.</i> , 2010)                             |
| 18 | Tannic acid                                                             | Leaves               | Tannins                       | Antimicrobial activity                                                                                                                  | (Dhanabalan <i>et al.</i> , 2008)                                 |
| 19 | Quercetin                                                               | Flower               | Flavonoid                     | Antibacterial activity                                                                                                                  | (Gnanasekaran <i>et al.</i> , 2017)                               |
| 20 | Iso-Quercetin                                                           | Flower               | Flavonoid                     | Antibacterial activity                                                                                                                  | (Ikewuchi, <i>et al.</i> , 2015)                                  |
| 21 | Luteolin                                                                | Flower               | Flavonoid                     | Antibacterial activity, Antioxidant, Anti-inflammatory, Anti-mutagenic<br>Antiallergic, antiandrogenic, anticancerous, radio-protective | (Dillard <i>et al.</i> , 2000; López-Lázaro, 2009)                |
| 22 | Kaempferol-3-O-rutinoside                                               | Leaves               | Flavanol                      | Hypotensive effect                                                                                                                      | (Ahmad <i>et al.</i> , 1993)                                      |
| 23 | Catechins                                                               | Leaves               | Flavonoid                     | Antimicrobial and Cardioprotective activity                                                                                             | (Dillard <i>et al.</i> , 2000)                                    |
| 24 | Epicatechin                                                             | Leaves               | Flavonoid                     | Antioxidant and Hypoglycemic activity                                                                                                   | (Dillard <i>et al.</i> , 2000; Chakravarthy <i>et al.</i> , 1982) |
| 25 | Nobiletin                                                               | Leaves               | Flavonoid                     | Antithrombotic, Anti-dyslipidemic, Anti-inflammatory activity                                                                           | (Ikemura, <i>et al.</i> , 2012)                                   |

|    |                                                      |                |                      |                                                                               |                                                          |
|----|------------------------------------------------------|----------------|----------------------|-------------------------------------------------------------------------------|----------------------------------------------------------|
| 26 | Diadzein and genistein                               | Leaves         | Isoflavones          | Antithrombotic, hypocholesterolemic, hypotensive effect                       | (Houston, 2005)                                          |
| 27 | Robinetin                                            | Leaves         | Flavone              | Chemopreventive agent                                                         | (Middleton <i>et al.</i> , 2000)                         |
| 28 | Akuammidine                                          | Leaves         | Alkaloid             | Antibacterial, antifungal activity                                            | (Pawar <i>et al.</i> , 2011)                             |
| 29 | Voacangine                                           | Leaves         | Alkaloid             | Anti-angiogenic and anti-mycobacterial activity                               | (Kim <i>et al.</i> , 2012; Rastogi <i>et al.</i> , 1998) |
| 30 | $\beta$ -Sitosterol                                  | Leaves, flower | Flavonoid            | Anti-inflammatory, antipyretic, immune-modulating, antineoplastic activities  | (Dillard <i>et al.</i> , 2000)                           |
| 31 | Ferulic acid                                         | Leaves         | Hydroxycinnamic acid | Antioxidant, anti-inflammatory, photoprotective, Cardio-protective activities | (Oksana <i>et al.</i> , 2012)                            |
| 32 | 3-O-methylquercetin-4'-O- $\beta$ -D-glucopyranoside | Aerial Part    | Flavonoid            | Antibacterial activity                                                        | (Mecina <i>et al.</i> , 2019)                            |
| 33 | Wogonin                                              | Flowers        | Flavonoid            | Antioxidant                                                                   | (Ma <i>et al.</i> , 2020)                                |
| 34 | Oroclin                                              | Flowers        | Flavonoid            | Antioxidant                                                                   | (Ma <i>et al.</i> , 2020)                                |
| 35 | Luteolin-4'-O- $\beta$ -glucopyranoside              | Aerial Part    | Flavonoid            | Antibacterial activity                                                        | (Mecina <i>et al.</i> , 2019)                            |

**Antifungal activity:** The effectiveness of antifungal agents has been studied by many researchers. Different methods of extracting *Tridax procumbens* have been by different researchers to cease the growth of different fungi (Baile and Parmar, 2023). The whole plant extract of *T. procumbens* was tested for antifungal activity against *Aspergillus niger*, a phytopathogenic fungus, and the leaf extract was found to have strong antifungal activity against *Fusarium oxysporum* (Bobbarala *et al.*, 2009). The disc

diffusion approach was used to assess the antifungal function of the plant decoctions. Three different fungi *Candida parapsilosis*, *Candida tropicalis*, and *Candida albicans* had been used to report the antifungal activity of essential oil extracted from *Tridax procumbens* of about 12-15mm zone of inhibition (Manjamalai *et al.*, 2012). Various sections of the plant like stem, root, flower, and leaf methanol extract have been prepared to show an important inhibitory effect against *Candida albicans*. At the concentration of



100mg/ml the inhibition zone ranging from 8mm to 13 mm. Antifungal activities were exhibited by the methanolic extract of roots against *Candida tropicalis* and *Candida glabrata* while methanol leaf extract displayed the susceptibility of *Candida albicans* and *Candida tropicalis*. Natural fungicidal agents can be employed to minimize the utilization of commercial chemical fungicides and mitigate their harmful consequences.

**Antioxidant activity:** Complete phenols, flavonoids, tannin content, and antioxidant vitamins are often used to describe plant antioxidant activity. Plant phenolics are well-known for their ability to scavenge free radicals and antioxidants, and these antioxidants not only protect lipids from oxidation but often have health benefits. They help in the prevention of diabetes (Nia *et al.*, 2003; Yen and Chen, 1995; Ebrahimzadeh *et al.*, 2010). The total phenol present in *T. procumbens* was formulated as gallic acid equivalent (GAE) which shows a high phenolic content of 12mg/g GAE. The unique bioactive compounds present in plant parts aided in the prevention of cancer, heart disease, and other age-related ailments. This was identified that by repairing damaged cells and by lowering cholesterol these bioactive compounds act as chemo-preventive agents. Antioxidants shield RBC membrane lipids from oxidative stress,

resulting in less hemolysis and red blood cells degradation, as shown by the osmotic fragility test. In a variety of fish species, the intake of different antioxidant compounds through diet bolstered antioxidant defenses and inhibited red blood cell hemolysis (Khan *et al.*, 2013; Gao *et al.*, 2016; Hoseini *et al.*, 2021). Antioxidant activity of *T. procumbens* was validated by ABTS [2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid)] and DPPH (2, 2-diphenyl-picrylhydrazyl hydrate) methods. The DPPH approach is a good way to determine in-vitro antioxidant activity. The ethanol extract's chloroform and ethyl acetate fractions demonstrated the highest efficacy in the DPPH method, with IC<sub>50</sub> values of 37.39 µg/ml.

**Hepatoprotective activity:** The liver contains all the enzymes which are involved in the detoxification mechanism, hence it is primarily detoxifying organ in the body. Significant protection was shown by the hepatoprotective property of leaves in the alleviation of D-Galactosamine/ Lipopolysaccharide-induced hepato-cellular injury. Because of their potential to damage liver cells, both these compounds have been proposed to be hepato-toxic. In humans, the lesions and the multifocal necrosis are similar which are produced by viral hepatitis and G-GalN respectively. The amino sugar inhibits transcription and, by extension, hepatitis protein

synthesis, and because of endotoxin toxicity, triggers hepatitis within 8 hours of ingestion.

**Wound healing activity:** It's a complex procedure that can help in restoring cellular structure and tissue layer. This plant's wound-healing mechanism entails a complex interaction between dermal and epidermal cells, the extracellular matrix, mediated angiogenesis, and plasma-derived proteins, all of which are regulated by cytokines and growth factors (Bhat *et al.*, 2007). The presence of replicating microorganisms can cause host injury and systemic toxicity within a wound or surrounding tissues which may be defined as a wound infection. The villagers apply the leaf juices of this plant to the wound portion basically to arrest bleeding from cuts and bruises in animals and to avoid such kinds of infections. In addition to promoting healing, the aqueous extracts of *T. procumbens* leaves were found to overcome the healing depression caused by steroids in male Wistar rats in experiments. The wound healing activity in a rat model was shown by plant extract with increased hexosamine and lysyl oxidase levels that are reported to stabilize the collagen fibers by increasing the cross-linking of collagen during the healing process. Protein synthesis of glycosamine glycan (GAGs) and the mRNA content were also increased by the plant extract; these are the main components of the extracellular

matrix in the granulation tissue. By comparing waterleaf decoction with the whole plant decoction, it was also effectively increasing the lysyl oxidase level but to a lesser degree. It was reported that the *Tridax procumbens* leaves extract promotes wound healing in both healthy and immune-compromised rats. Significant wound healing activity was shown by the ethanolic extract in gel-based formulation (Bhat *et al.*, 2007). The whole plants aqueous and ethanol extracts are utilized for treating excision and incision wounds. *T. procumbens* extracts show indirect corticotropic effects in the excision wound healing process. In this process, the wound was treated with the plant extract within 15 days. By using the aqueous and ethanol extracts of the entire plant, the tensile strength of collagen fibers and the rate of epithelialization are enhanced in the treatment of excision and incision wounds (Mundada and Shivhare, 2010; Ingle, *et al.*, 2014; Bhat *et al.*, 2007). Whereas the incision wound was treated with plant extract for 14 days. A significant increase in hydroxyproline, hexosamine, and collagen was shown by *T. procumbens* which results in effective wound healing activity (Talekar *et al.*, 2012). Bacterial cellulose films also have wide applications in the biomedical fields which include skin burn transfer, wound healing, and scaffold tissue engineering. It is an excellent biopolymer that requires the implementation of

antibiotics to show antimicrobial activity. In a study, it was investigated that the cuts and wounds were also treated with *T. procumbens* by the Kuruma tribe of Kerala's Wayanad district and by Paliyar tribes in Tamil Nadu. In the Wayanad district, the Kurumas are the most prominent scheduled tribes. Woodcutting and the processing of minor forest items were the Kurumas' primary occupations. They are mainly farm workers, with some cultivators thrown in for good measure. Skin diseases, diarrhoea, malnutrition, jaundice, worm infections, and fever are some of the most common health problems they face (Hema, *et al.*, 2006). Paliyars are the indigenous people of Palani hills. Paliyars are generally illiterate and they speak Tamil when compared with the other tribal communities they constitute a relatively small group in Tamil Nadu (Kamatchi & Subramaniam, 2019). The leaves paste of *T. procumbens* is applied to the wounds by the people of both these tribes.

**Anti-diabetic activity:** *T. procumbens* leaves extract (aqueous, ethanolic, and methanolic) exhibited anti-diabetic activity which is significantly decreased in the blood glucose level. Alloxan-induced diabetes in rats was taken as a model for testing anti-diabetic activity (Bhagwat *et al.*, 2008). The beta cells of the pancreas producing insulin are destroyed by alloxan and cause diabetes. Alloxan causes necrosis by being

selectively toxic to beta cells. The ethanolic extract decreased blood sugar levels in diabetic rats by 10% to 17% but had no effect on normal rats' fasted blood sugar levels. The methanolic extract of 50% of acute and subchronic doses was administered orally to reduce the fasting blood glucose levels in diabetic rats. In normal rats, it has no effect on blood sugar levels (Salahdeen *et al.*, 2004; Pareek *et al.*, 2009). At 200mg/kg of body weight a dose of water, alcoholic and petroleum ether decoctions of leaves were orally administered in animals for about seven days and it was reported that the blood glucose level was significantly reduced by the water and alcoholic decoctions in animal model whereas the petroleum ether extract exhibits a feeble anti-diabetic effect.

**Anti-inflammatory activity:** This activity was significantly shown by the aqueous, ethanol, methanol, and ethyl acetate extracts of *T. procumbens* by hinder the actions of inflammatory mediators such as serotonin, prostaglandins, histamine, and bradykinin (Das *et al.*, 2009). The corticotrophic influence was also responsible for the anti-inflammatory effect, as shown by the weight gain. Exudates length, leukocyte migration, granuloma tissue, edoema fluid, and glutamyl transpeptidase were all significantly reduced by different extracts of the plant, indicating that it has an anti-inflammatory effect. The anti-inflammatory action of this plant extract was

assessed on carrageenan-induced paw edema along with the standard drug, Ibuprofen. When we treat the *Tridax* extract with a standard drug Ibuprofen it was observed that there was an increase in the inhibition of edema. Quercetin, a flavonoid compound, exhibits analgesic and anti-inflammatory properties. Additionally, it may play a role in inhibiting the anti-allodynic effect and inflammatory pain associated with neuropathic pain induced by chronic constriction injury (CCI) (Margaret *et al.*, 1998; Sawant *et al.*, 2014). Alkaloids and flavonoids were used to combat reactive oxygen species, which were linked to the pathogenesis of inflammation and other diseases (Prabhu *et al.*, 2011).

**Anti-cancerous activity:** The anti-metastatic activity was shown by the aqueous leaves extract of *T. procumbens* with essential oils in the C57BL/6 (B16 F-10 melanom cell lines) mice which was developed by lung cancer, and which was evidenced by deterring the increase in WBCs, hemoglobin count, and in body weight. The active compounds present in it mainly belong to monoterpene family which involves sabinene,  $\alpha$ -pinene,  $\beta$ -pinene, and phellandrene. The Terminal deoxynucleotidyl transferase UTP nick end

labeling (TUNEL) assay was used to analyze the expanded expression of caspase-3 and p53 which was used to characterize the monoterpenes. By using the plant-derived compounds the cytotoxicity was determined against the human lung cancer cell line by MTT assay. 90% reduced cell viability was shown by the compounds. The dose- dependent bioactivities and IC<sub>50</sub> values of *Tridax procumbens* from current experimental investigations are compared in table 5, which also highlights important active ingredients and assay methods. The studies summarize that *T. prhavembens* has multifunctional bioactivity in a variety of metabolic pathways. By scavenging radicals, inhibiting enzymes (COX, LOX, and iNOS), and suppressing the NF- $\kappa$ B pathway, flavonoids and polyacetylenes are the main source of antioxidant and anti-inflammatory actions. The therapeutic value of alkaloidal and phenolics fractions for the development of phytopharmaceuticals are highlighted by their antimicrobial potency at sub-milligram MIC levels. Potential for biocompatible therapeutic materials is suggested by the cytotoxic and wound healing properties, especially when combined with polymeric matrix like PVA or chitosan for prolonged release.

**Table 5: Dose-dependent data of *Tridax procumbens*, including IC<sub>50</sub> ranges, effective dosages and important active ingredients documented in recent in-vitro assays**

| S. No. | Bioactivity Type                  | Extract                           | Assay                                                              | IC50 or Effective dose (µM or µg/ml)             | Key Active constituents                        | References                      |
|--------|-----------------------------------|-----------------------------------|--------------------------------------------------------------------|--------------------------------------------------|------------------------------------------------|---------------------------------|
| 1      | Antioxidant                       | Fractionated methanolic Extract   | ABTS, FRAP, TRP assays                                             | 14.7-93.8 µg/ml                                  | Total flavonoids content correlated positively | Sagheer <i>et al.</i> , 2024    |
| 2      | Antibacterial                     | Methanolic floral extract         | <i>E. coli</i> , <i>S. aureus</i> MIC                              | 100-200 µg/ml                                    | Alkaloids, flavonoids, β-sitosterol            | Jindal <i>et al.</i> , 2012     |
| 3      | Antioxidant                       | Ethanolic extract                 | DPPH, hydroxyl radical scavenging                                  | 0.12-0.15 µg/ml                                  | Phenolics, Flavonoids (Quercetin, luteolin)    | Ahamad <i>et al.</i> , 2023     |
| 4      | Antifungal                        | Methanolic root and leave extract | <i>Candida albicans</i> , <i>Aspergillus niger</i> inhibition zone | 8-15 mm (100 mg/ml)                              | Terpenoids, phenolics, essential oils          | Jindal <i>et al.</i> , 2012     |
| 5      | Anti-inflammatory                 | Polyacetylene fraction            | LPS- induced no inhibition                                         | 15.9-20.4 µM                                     | Polyacetylenes                                 | Tan <i>et al.</i> , 2024        |
| 6      | Anticancer                        | Essential oil                     | MTT assay                                                          | 50 µg/ml                                         | Monoterpenes, sesquiterpenes                   | Manjamalai <i>et al.</i> , 2012 |
| 7      | Anticancer/ Cytotoxic             | Polyacetylene fraction            | K562 leukemia cells                                                | 2.6-17.9 µM                                      | Tridaxin B, tridaxin F                         | Tan <i>et al.</i> , 2024        |
| 8      | Wound Healing                     | Ethanolic leaf extract            | Scratch assay                                                      | Dose-dependent at 50-200 µg/ml                   | Flavonoids, tannins                            | Cheriyian <i>et al.</i> , 2025  |
| 9      | Antioxidant and anti-inflammatory | <i>T. procumbens</i> chitosan gel | DPPH, BSA/EA denaturation assays                                   | Dose-dependent upto 50 µL for maximum inhibition | Phenolic acids, leaf extract bioactives        | Nandita <i>et al.</i> , 2024    |

**Immunomodulatory activity:** The immunomodulatory effect was shown by the ethanolic decoction of leaves of the *T. procumbens* on Albino rats which was prescribed with *Pseudomonas aeruginosa* and for the same proliferation was also inhibited (Oladunmoye *et*

*al.*, 2006). This property helps in the enhancement in the uptake of particulate matter by using the phagocytes and by increasing the number of leukocytes, splenic leukocytes, plasma cells and increase in the phagocytic index this also helps in the stimulation of cell-mediated immune response. The ethanolic extract majorly contains an active

component named 'sesquiterpene lactone' which is basically known to induce T-cell mediated hypersensitivity or the delayed-type hypersensitivity reaction. As there was an increase in the concentration of hemagglutination antibody it was observed that the humoral immune response was also stimulated. It also identifies the components to modulate the immune system for both cell-mediated and hormonal components by saponin fraction, flavonoids, and phytoconstituents as the immunomodulatory potential of *Tridax procumbens*.

**Cardiovascular effects:** An anesthetized Sprague-Dawley rat was taken as a model animal to examine the cardiovascular effects of *Tridax* juicy leaf extract. The average value of arterial blood pressure was declined due to a significant dose-dependent water decoction of this plant. There was a reduction in the heart rate when the higher dose was applied, and the heart rate remains unchanged when the lower dose was given. The animals were pre-treated with atropine sulfate to inhibit the hypotensive effect. The activation of muscarinic cholinergic reporters was activated for action mechanisms (Salahdeen *et al.*, 2004).

**Anti-leishmanial activity:** "Chiclerous ulcer is another name for cutaneous leishmaniasis which is caused by the promastigotes of *Leishmania Mexicana*, and a biochemical oxylipin namely

[3S]-16,17-Didehydrofalcariinol found to be an active compound in *T. procumbens* which shows considerable anti-leishmanial activity against it (Martin-Quintal *et al.*, 2009; Gamboa-Leon *et al.*, 2014).

**Repellency activity:** Steam distillation was used for the extraction of essential oils from the *T. procumbens* leaves. In the mosquito, the malarial parasite *Anopheles stephensi* was enclosed for its tropical repellent effect and was treated by the extracted essential oil. Three different concentrations were taken for the determination of all essential oils and out of all these the essential oils from *Tridax* possess a comparatively high repellency effect and it was concluded that at 6 percentage concentration the *Tridax* promising as a repellent against *Anopheles stephensi*. Different pharmacological effects were reported by the decoctions of *Tridax procumbens*, Lorkes method was used to study the acute toxicity (Lorke, 1983). The acute toxicity of the decoction compounds is determined by whether they are circulated to the target organ or transferred directly into the bloodstream. This determination is typically based on intraperitoneal administration. The Aspartate amino transaminase (AST) enzymes level and glucose levels decreased while urea, Alanine amino transaminase (ALT),  $\text{Na}^+$  and  $\text{K}^+$  concentration increases (Abubakar *et al.*, 2012). Toxicity studies of this plant for the crude ethyl



acetate decoction concluded that the test animal's liver was significantly affected at all the doses.

### **Mechanistic pathway and biochemical interactions of major phytochemical constituents**

The pharmacological activities of *T. procumbens* are closely linked to its rich phytochemical composition, wherein major constituents such as flavonoids, alkaloids, terpenoids, phenolics acids, and sterols contribute to its therapeutic efficacy through multiple biochemical mechanisms. Flavonoids such as luteolin, kaempferol, and Quercetin mitigate oxidative stress by scavenging reactive oxygen species (ROS). Additionally, by inhibiting key enzymes including lipoxygenase (LOX) and cyclooxygenase-2 (COX-2), they suppress the biosynthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes. This combined antioxidant and anti-inflammatory mechanism underlines many of the traditional therapeutic applications of *T. procumbens* in wound healing and inflammation management (Andriana *et al.*, 2019). Saponins, sesquiterpenes, and flavonoids modulate immune function by enhancing leucocyte proliferation and antibody synthesis. Additionally, they promote collagen formation and fibroblast activity, thereby accelerating tissue regeneration and repair (Baile *et al.*, 2024). In conclusion, the broad pharmacological potential of *T. procumbens* arises

from the synergistic interactions of its diverse bioactive constituents, which collectively regulate oxidative stress, inflammation, microbial activity, metabolic enzyme function, and immune responses through defined molecular pathways. This comprehensive mechanistic insight reinforces its therapeutic significance and provides a strong foundation for future research and biotechnological exploitation.

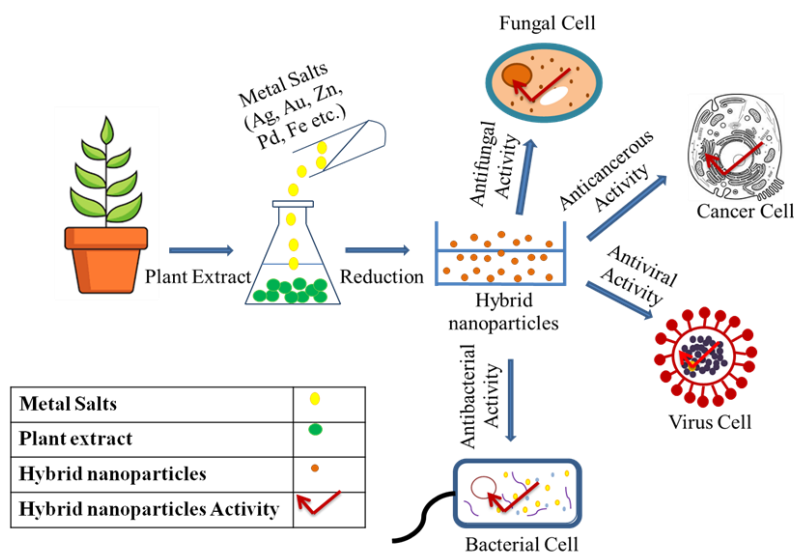
### **Preparation of nanoparticles:**

Nanotechnology is one of the most fascinating areas, involving the synthesis and modification of atoms and molecules at the nanoscale. The size of nanoparticles varies between 1 and 100 nm. All the fundamental features (chemical, physical, and biological) change considerably at the atomic and molecular levels as compared to their bulk counterparts due to their small size. A variety of conventional and biological methods are available for the green synthesis of nanoparticles. Only a few studies have been done on the antibacterial and antioxidant efficacy of AgNPs produced by this plant (Bhati--Kushwaha *et al.*, 2014; Dhanalakshmi *et al.*, 2012). Considering the medicinal value of this plant, Ag/Cu<sub>2</sub>O nanocomposites are prepared by the leaf extracts of *T. procumbens*. Leaf extracts help silver and copper ions (Ag<sup>+</sup> and Cu<sup>2+</sup> ions) to reduce into Ag<sup>0</sup> and Cu<sup>0</sup> respectively or it acts as a reducing agent. Silver nanoparticles which are spherical shaped,



pure, and crystalline synthesized from the succulent leaf abstract of this plant and they exhibit anti-microbial activity and also inhibit different

gram negative, gram positive bacterial, and fungal strains (Figure 4).



**Figure 4: Green Synthesis of hybrid nanoparticles from *Tridax procumbens* extract and its application**

### ***Anti-inflammatory effect of crude extract and nanoparticles***

*In-vitro* denaturation of protein was shown high by *T. procumbens* when egg albumin was used as protein. Higher anti-inflammatory activity was shown by the crude extract of this plant when correlated with diclofenac sodium which was taken as normal. It was noticed that with the increase in the concentration of extract nanoparticle formulation activity also goes higher.

It resulted by the researchers that the nanoparticle formulation shows a higher percentage inhibition.

### ***Anti-oxidant property for *T. procumbens* nanoparticles***

This indicates that the polymeric nanoparticles of the extracts have a higher antioxidant activity than the crude extracts, which may be due to their increased surface area and better dispersion in the

system. The antioxidant activity of the nanoparticles can protect cells from oxidative stress and prevent the development of various diseases associated with free radical damage. Therefore, the use of polymeric nanoparticles of plant extracts may be a promising approach for developing natural antioxidant agents for therapeutic applications. However, more studies are needed to evaluate their safety and efficacy in vivo. There was a decrease in the antioxidant activity as we increased the concentration of nanoparticles extract. Due to the presence of the flavonoids, there was an increase in the *T. procumbens* formulations which shows antioxidant activity.

## Conclusion

*Tridax procumbens* represents a valuable medicinal plant with diverse pharmacological potentials, including antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and wound-healing properties. Its broad therapeutic efficacy is attributed to the presence of various bioactive compounds, while biotechnological approaches such as micropropagation and callus culture offer sustainable means for metabolite production and germplasm conservation. However, existing studies are largely limited to preliminary in vitro or in vivo analyses, with insufficient molecular characterization and clinical validation of specific

compounds or mechanisms of action. The lack of standardized methodologies also restricts reproducibility and translational application. Future research should emphasize bioassay-guided isolation and molecular identification of active constituents, supported by genomic, transcriptomic, and metabolomic studies to unravel biosynthetic pathways. Integrating computational modeling, nanotechnology, and well-designed clinical trials will be crucial to advance *T. procumbens* from traditional use to evidence-based therapeutic development.

**Conflict of Interest** – The authors declare that they have no conflicts of interest.

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