BIOACTIVE PROFILING AND ANTIMICROBIAL EFFICACY OF *Tridax procumbens*: A GC-MS AND PHYTOCHEMICAL APPROACH

DR. T. SHARMILA RAJ[1]*, SUBHIKSHA M [2]

[1], Department of Biotechnology, Sri Shakthi Institute of Engineering and Technology, Sri Shakthi Nagar, L & T bypass road, Chinniyampalayam Post, Coimbatore, Tamil Nadu 641062, India.

ABSTRACT

Tridax procumbens L. (Asteraceae) is a medicinal plant traditionally valued for its wound-healing, anti-inflammatory, and medicinal properties related to gastrointestinal, hepatic, and respiratory diseases. This study analyzed its phytochemical and chemical composition using gas chromatography-mass spectrometry (GC-MS), as well as its antimicrobial efficiency. Leaves were air-dried, ground, and extracted using acetone with ultrasonication. Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, and cardiac glycosides; however, tannins and steroids were completely absent.

GC–MS analysis indicated the presence of a number of key bioactive components, including phytol, stigmasterol, α-linolenic acid, γ-tocopherol, squalene, pectolinaringenin, campesterol, as well as a range of heterocyclic derivatives known to possess anti-inflammatory or immunomodulatory activity. Antimicrobial testing using the agar well diffusion method indicated moderate antibacterial activity against *Bacillus paralicheniformis*, which elicited a consistent 12 mm inhibition zone at 50–100 µL compared to 16 mm for the gentamicin control. No antifungal activity was observed against *Candida utilis.*

These results further indicate the medicinal importance of T. procumbens and its potential for valued plant-based sources of antibacterial drugs, for which further pharmacological validations are called for.

Keywords: Phytochemical evaluation, GC – MS analysis, immunomodulatory properties, bioactive compounds, plant-based antibacterial agents

INTRODUCTION

Medicinal plants have historically constituted an essential reservoir of therapeutic compounds, significantly influencing the evolution of both traditional and contemporary medical practices. Within the extensive array of medicinal vegetation, *Tridax procumbens* L., a species classified under the family Asteraceae, has attracted substantial scholarly attention due to its multifaceted ethnomedicinal uses and pharmacological capabilities. Commonly referred to as Coat Buttons or Tridax Daisy, *T. procumbens* is a prostrate or creeping annual herbaceous organism, extensively prevalent in tropical and subtropical locales, notably in India, Southeast Asia, Africa, and South America (Kumar et al., 2019). It is frequently located in disturbed ecosystems such as roadsides, wastelands, and open grasslands, and is often regarded as a weed within certain agricultural frameworks. Notwithstanding its classification as a weed, this plant has been historically employed in traditional medicinal systems, including Ayurveda and folk remedies, to address a diverse array of health conditions.

Figure 1: *Tridax procumbens*

Scientific Classification of *Tridax procumbens* (The Plant List, 2013):
Kingdom: Plantae
Clade: Angiosperms
Clade: Eudicots
Clade: Asterids
Order: Asterales
Family: Asteracea
Genus: Tridax
Species: *Tridax procumbens* L.

The morphology of the plant is distinguished by ovate to lanceolate leaves exhibiting irregular margins, pubescent stems that establish roots at the nodes, and solitary capitula featuring yellow disc florets alongside white or pale-yellow ray florets. It exhibits flowering throughout the entire year and propagates via both seeds and vegetative means (Verma et al., 2018). Traditionally, the entirety of the plant, particularly the leaves, has been utilized for the management of wounds, cuts, insect stings, and hemorrhages, attributable to its wound-healing and hemostatic attributes. In various rural populations across India, the fresh extract of *T. procumbens* leaves is applied to treat open wounds or consumed to alleviate stomachaches, diarrhea, liver ailments, and respiratory infections (Patel et al., 2011; Meena et al., 2020). The extensive traditional application of *T. procumbens* underscores its medicinal significance and has spurred scientific investigation into its phytochemical constituents and pharmacological properties.

The rise of multidrug-resistant pathogens has prompted the search for novel antimicrobial agents from natural sources. *T.procumbens* is a notable phytopharmaceutical resource with diverse bioactive properties. Its accessibility, historical safety, and extensive pharmacological range position it favourably for further investigation in herbal drug, nutraceutical, and functional food development.

Consequently, this study aims to thoroughly examine the phytochemical profile, GC-MS characteristics, and antimicrobial efficacy of *Tridax procumbens* leaf extracts. By merging traditional knowledge with contemporary

analytical methods, the research seeks to substantiate the plant's therapeutic potential and enhance the evidence base for ethnobotanical resources in drug discovery.

MATERIALS AND METHODS

Collection And Preparation Of Plant Material

Fresh foliage of *Tridax procumbens* was procured from multiple sites in proximity to Avinashi, located within the Tiruppur District of Tamil Nadu, India, and underwent extensive washing with distilled water to eliminate any adhering particulates and contaminants. The purified plant material was subsequently shade-dried at ambient temperature to safeguard thermolabile phytochemicals. The desiccated leaves were then finely milled into a powder utilizing a mechanical grinder and were stored in hermetically sealed containers to avert moisture intrusion and degradation of bioactive constituents.

Acetone Extraction Via Ultrasonication

A precisely measured quantity of the desiccated leaf powder (for example, 5 g or 10 g) was combined with a suitable volume of analytical-grade acetone (for instance, 50 mL or 100 mL). This amalgamation was subjected to ultrasonication in an ultrasonic bath or via a probe sonicator operating within a frequency range of 20–40 kHz for a duration of 30 to 60 minutes. The ultrasonic waves induced cavitation bubbles that disrupted cellular membranes, thereby facilitating the release of intracellular phytochemicals into the acetone solvent. The temperature was meticulously regulated to remain below 40°C to prevent the degradation of sensitive compounds.

Filtration And Concentration

Subsequent to sonication, the mixture was either filtered using Whatman filter paper or subjected to centrifugation at 10,000 rpm for 10 minutes to isolate the supernatant. The acetone present in the filtrate was subsequently evaporated under reduced pressure utilizing a rotary evaporator, resulting in the acquisition of the crude acetone extract, which was stored at 4°C until further application.

QUALITATIVE PHYTOCHEMICAL TESTS

The crude acetone extract underwent qualitative screening for significant secondary metabolites employing standardized methodologies and specific chemical reagents

Alkaloids (Wagner’s Test):

Wagner’s reagent (iodine in potassium iodide) was introduced to the extract. The formation of a reddish-brown precipitate signified the presence of alkaloids.

Flavonoids (NaOH Test):

A few drops of sodium hydroxide were incorporated into the extract. The emergence of a yellow coloration, which dissipated upon acidification, confirmed the presence of flavonoids.

Phenolic Compounds (Ferric Chloride Test):

The addition of ferric chloride ($FeCl_3$) to the extract resulted in the formation of a blue, green, or purple complex, indicating the presence of phenolic compounds.

Tannins (Ferric Chloride Test):

The identical ferric chloride reagent was employed; the manifestation of a dark blue or green hue suggested the presence of tannins, while the absence of color change indicated their nonexistence.

Steroids (Liebermann–Burchard Test):

A mixture comprising acetic anhydride and concentrated sulfuric acid was added. The appearance of a green, blue, or brown coloration confirmed the presence of steroids.

Glycosides (Keller – Kiliani Test):

A mixture of glacial acetic acid, ferric chloride, and concentrated H_2SO_4 was introduced to the extract. The formation of a brown ring at the interface indicated the presence of cardiac glycosides.

GC–MS ANALYSIS

The gas chromatography-mass spectrometry (GC-MS) analysis of Tridax procumbens provides a comprehensive phytochemical profile, revealing the plant's diverse secondary metabolites. This high-resolution analytical technique enabled the identification and quantification of bioactive compounds, categorizing them into distinct structural classes based on their retention times and mass fragmentation patterns. By correlating these chemical constituents with documented biological activities, this analysis offers critical insights into the mechanistic basis of T. procumbens' therapeutic potential while establishing a foundation for future phytochemical and pharmacological studies.

ANTI–MICROBIAL ACTIVITY

Test Sample and Microorganisms

The sample was examined for its antimicrobial efficacy against *Bacillus paralicheniformis* (a bacterial strain) and *Candida utilis* (a fungal strain). The bacterial strain was cultivated on Luria–Bertani agar (LB agar), whereas the fungal strain was grown on Potato Dextrose Agar (PDA).

Agar Well Diffusion Assay

The determination of antimicrobial activity was conducted utilizing the agar well diffusion methodology. In summary, the suitable culture medium was formulated and sterilized, subsequently cooled to approximately 45 °C. An aliquot of 1 mL of microbial inoculum was incorporated into 15–20 mL of molten agar and gently mixed prior to being poured into sterile Petri dishes. Following solidification at ambient temperature, 8 mm diameter wells were aseptically created in the agar using a sterile 1000 µL pipette tip.

Various volumes (50 µL, 75 µL, and 100 µL) of the test sample were introduced into the wells. In the bacterial assay, 100 µL of Gentamycin (50 µg/mL) was utilized as the positive control, whereas 100 µL of Fluconazole (500 mg/mL) was employed as the positive control for the fungal assay. The plates were incubated at 30 °C for a duration of 18–24 hours, dependent upon the optimal growth conditions of the organism. Upon completion of the incubation period, the diameters of the zones of inhibition (ZOI) were measured in millimeters (mm).

Ethical Statement

No human or animal subjects were involved in this study. Hence, ethical approval was not required.

RESULTS

Phytochemical Analysis

The phytochemical screening of *Tridax procumbens* revealed the presence of several bioactive compounds, which are crucial for its medicinal properties. The analysis was conducted using standard chemical tests, and the results indicated the presence of alkaloids, flavonoids, phenolic compounds, and glycosides. However, tannins and steroids were not detected in the tested sample.

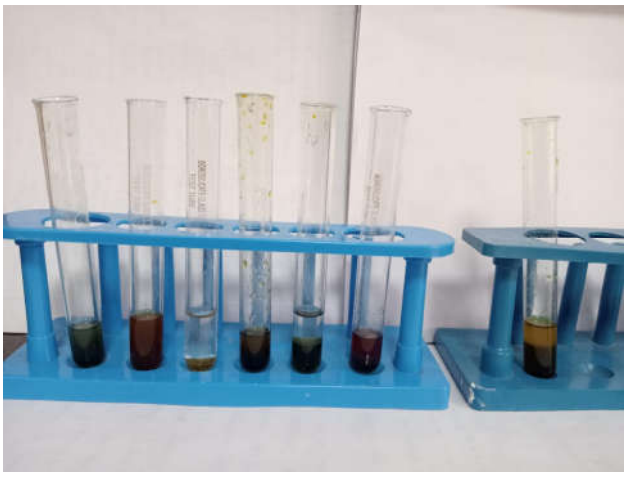

Figure 2: Phytochemical analysis of *T.procumbens*

The summarized results are presented in the table below:

Table 1: Phytochemical Results Of *T.procumbens*

Phytochemical	Test Used	Result
Alkaloids	Wagner's Reagent Test	Present
Flavonoids	Sodium Hydroxide Test	Present
Phenolic Compounds	Ferric Chloride Test	Present
Tannins	Ferric Chloride Test	Absent
Steroids	Liebermann-Burchard Test	Absent
Glycosides	Keller-Kiliani Test	Present

GC-MS ANALYSIS

In order to effectively ascertain the precise percentage concentration of a specific compound through the sophisticated analytical technique known as Gas Chromatography-Mass Spectrometry (GC-MS) analysis, it is imperative that one initially acquires and meticulously analyses the peak areas that correspond to all identified compounds, a process that is systematically delineated by the advanced capabilities of the GC-MS software, such as the renowned Agilent Mass Hunter. The Total Ion Chromatogram (TIC) serves as an invaluable tool for the comprehensive integration of the cumulative peak areas, allowing for a more nuanced understanding of the compound distribution within the sample matrix.

Following this initial analytical step, the relative percentage of each compound can then be computed with precision by utilizing the established formula, which is essential for ensuring the reliability and accuracy of the results obtained.

$$\text{Relative } (\%) = \frac{(\text{Peak area of Compound})}{(\text{Total area of all peaks})} \times 100$$

This calculation not only effectively quantifies the fraction of a given compound in relation to the overall composition of the sample, but it also facilitates a more thorough and accurate biochemical characterization, which is indispensable for advancing analytical research and contributing to the broader field of scientific inquiry.

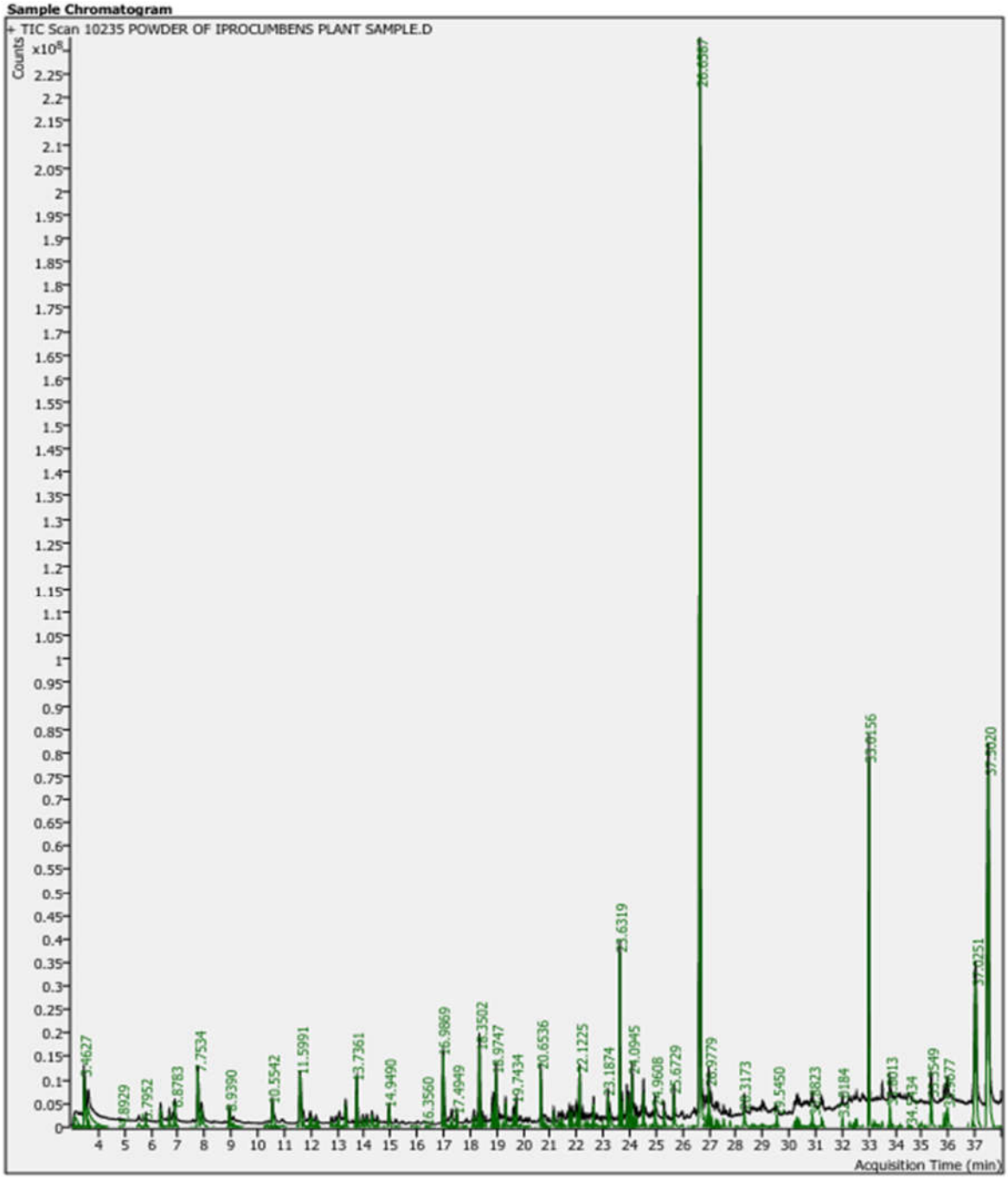

Figure 3*:* GC-MS chromatogram o*f T.procumbens*

Table 2: Bioactive Compounds And Their Immunomodulation Activity

Compound	RT (min)	Formula	Mechanism of Immunomodulation
Phytol	26.6587	$C_{20}H_{40}O$	↑ IL-10 (anti-inflammatory cytokine), ↓ TNF-α
Stigmasterol	37.502	$C_{29}H_{48}O$	Modulates Th1/Th2 balance, ↓ IL-6
α-Linolenic acid	26.9779	$C_{18}H_{30}O_2$	Converts to EPA/DHA to ↓ NF-κB signaling
γ-Tocopherol	34.9765	$C_{28}H_{48}O_2$	↓ Pro-inflammatory cytokines (IL-1β, IL-6)
Squalene	33.0156	$C_{30}H_{50}$	Adjuvant effect → ↑ Antibody production
Pectolinaringenin	36.9343	$C_{16}H_{12}O_5$	Flavonoid to ↓ TLR4/NF-κB pathway

Campesterol	37.0251	$C_{28}H_{48}O$	↓ IFN-γ (Th1 response modulator)
2,4-Di-tert-butylphenol	19.3356	$C_{14}H_{22}O$	Suppresses LPS-induced inflammation
Q Neophytadiene	23.6319	$C_{20}H_{38}$	↓ COX-2/PGE2 (anti-inflammatory)
Rauwolscine methyl ester (Yohimban derivatives)	37.9756	$C_{24}H_{30}N_2O_5$	↓ α2-Adrenergic receptor → immune cell regulation
Benzothiazoles	23.4174	C_7H_5NS	↑ Antimicrobial peptides (e.g., defensins)
1,2,4-triazole derivatives.	21.6437	$C_4H_7N_3OS$	↑ Macrophage phagocytosis

ANTI-MICROBIAL ACTIVITY

The antimicrobial efficacy of *Tridax procumbens* (acetone extraction) against *Bacillus paralicheniformis* and *Candida utilis* was evaluated by quantifying the ZOI generated at varying concentrations (Table 3). In the case of *Bacillus paralicheniformis*, the sample consistently generated inhibition zones of 12 mm at 50 µL, 75 µL, and 100 µL, in contrast to the 16 mm observed for the Gentamycin positive control. Conversely, the sample demonstrated no observable antifungal activity against *Candida utilis* at any of the concentrations tested, while the Fluconazole positive control yielded a ZOI of 26 mm.

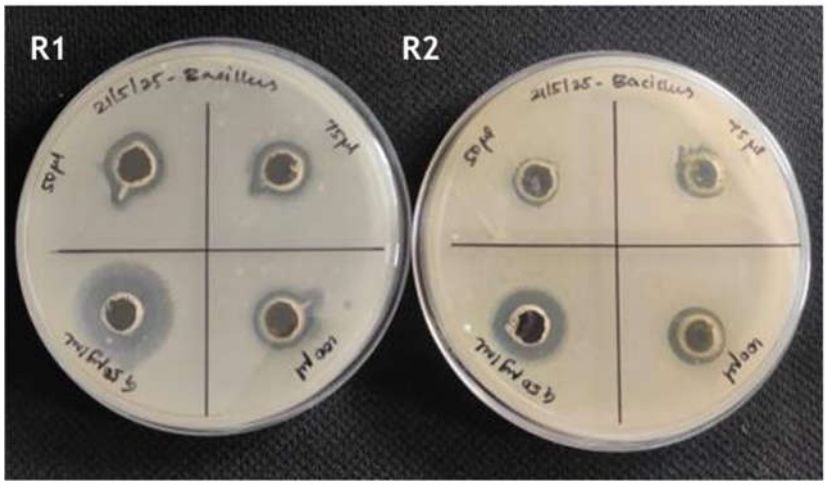

Figure 4: Anti-Bacterial activity of *Tridax procumbens* against *Bacillus paralicheniformis*

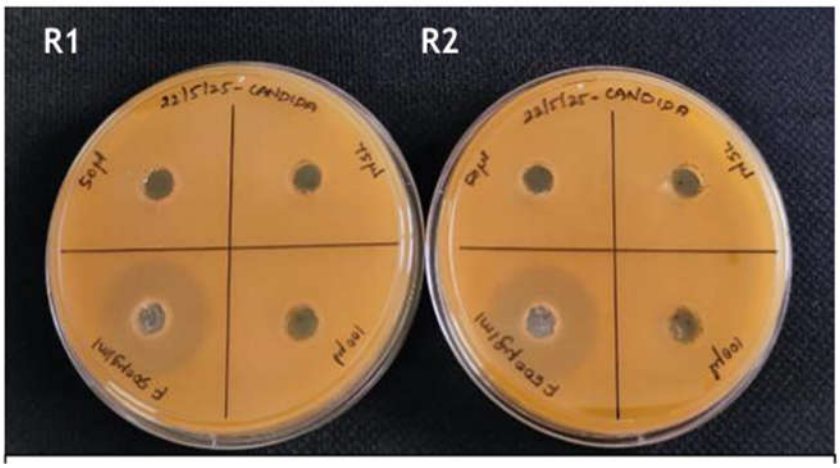

Figure 5: Anti-fungal activity of *Tridax procumbens* against *Candida utilis*

Table 3: Antimicrobial Activity of *Tridax Procumbens* (Acetone Extract) Against Test Organisms

S.No	Organism	Zone of Inhibition			
		50µL	75µL	100µL	Positive Control
1	*Bacillus paralicheniformis*	12 mm	12 mm	12 mm	16 mm
2	*Candida utilitis*	No inhibition	No inhibition	No inhibition	26 mm

DISCUSSION

The present study elucidated the phytochemical composition, antimicrobial potential, and immunomodulatory implications of *Tridax procumbens* through acetone extraction, qualitative biochemical tests, and GC–MS analysis. The extraction process facilitated the isolation of a broad range of bioactive metabolites, validating the plant's long-recognized ethnomedicinal relevance.

The qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolic compounds, and glycosides—classes of compounds well-documented for their antioxidant, antimicrobial, and immune-enhancing effects. The absence of tannins and steroids suggests that the acetone solvent system selectively extracted moderately polar compounds, which are typically abundant in flavonoids and phenolics. These findings corroborate earlier studies indicating that *T. procumbens* extracts are rich in polyphenolic compounds contributing to their pharmacological potential.

The GC–MS analysis further substantiated the presence of several key bioactive molecules such as phytol, stigmasterol, α-linolenic acid, γ-tocopherol, squalene, and pectolinaringenin, among others. Many of these compounds possess well-established immunomodulatory properties, including the suppression of pro-inflammatory mediators (e.g., TNF-α, IL-6) and the enhancement of anti-inflammatory cytokines (e.g., IL-10). For instance, phytol and γ-tocopherol are recognized antioxidants that mitigate oxidative stress and regulate cytokine release, while stigmasterol and campesterol are phytosterols known to modulate immune cell signaling and inflammation. The detection of compounds such as 2,4-di-tert-butylphenol and benzothiazole derivatives also implies potential antimicrobial and antioxidant contributions.

The antimicrobial assay results demonstrated that the acetone extract of *T. procumbens* exhibited measurable inhibitory activity against *Bacillus paralicheniformis*, producing consistent zones of inhibition (12 mm) across different concentrations. This moderate antibacterial effect could be attributed to the synergistic interaction of phenolic compounds and flavonoids disrupting bacterial cell membranes and interfering with enzyme systems. However, the extract showed no inhibitory effect against *Candida utilis*, suggesting limited antifungal potential under the tested conditions. This selective antimicrobial activity indicates that the phytochemicals in *T. procumbens* are more effective against Gram-positive bacteria than fungal species, aligning with previously reported antibacterial dominance in similar extracts.

Collectively, the findings highlight the pharmacological promise of *T. procumbens* as a source of immunomodulatory and antimicrobial agents. The diverse bioactive compounds detected via GC–MS provide a

biochemical basis for its traditional medicinal use and encourage further exploration through bioassay-guided fractionation and in vitro/in vivo validation of its therapeutic efficacy.

CONCLUSION

The comprehensive analysis of *Tridax procumbens* acetone extract revealed the presence of multiple biologically active phytoconstituents, including alkaloids, flavonoids, phenolic compounds, and glycosides, as confirmed through qualitative and chromatographic analyses. The GC–MS profiling identified several immunomodulatory compounds, notably phytol, stigmasterol, and γ-tocopherol, which are associated with anti-inflammatory and immune-regulatory mechanisms.

The extract demonstrated moderate antibacterial activity against *Bacillus paralicheniformis*, indicating potential for natural antimicrobial agent development, although it exhibited no antifungal efficacy against *Candida utilis*. These outcomes suggest that *T. procumbens* contains a spectrum of compounds capable of influencing both microbial and immune systems, thereby supporting its ethnopharmacological applications.

In conclusion, this study underscores *T. procumbens* as a valuable medicinal plant with significant potential for therapeutic exploitation. Future research should focus on isolating and characterizing the active principles, evaluating their mechanisms of action, and assessing their efficacy in advanced pharmacological models to establish a scientific foundation for their clinical use.

ACKNOWLEDGEMENT

The authors sincerely express their gratitude to the Department of Biotechnology, Sri Shakthi Institute of Engineering and Technology, Coimbatore, for providing the laboratory facilities and technical support required to complete this research work. The authors also thank Dr. T. Sharmila Raj for her continuous guidance, supervision, and valuable scientific insights throughout the study.

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