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Evaluation of in vitro cytotoxicity and anti oxidant activity of Tridax Procumbens

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INTODUCTION

Tridax procumbens, a medicinal plant species belonging to the Asteraceae family, has garnered significant attention from researchers worldwide due to its immense antibacterial and antifungal potential, which has been documented through numerous scientific investigations over recent decades. This remarkable plant, commonly known as coat buttons or tridax daisy, is widely distributed across tropical and subtropical regions globally, with particularly extensive presence throughout Asia, Africa, and Australia, where it thrives in diverse environmental conditions ranging from roadsides and agricultural fields to wastelands and urban areas. One of the most notable characteristics of Tridax procumbens from both ecological and medicinal perspectives is its availability in almost all seasons in almost all parts of the country where it grows, ensuring a consistent and accessible supply of plant material for traditional medicine practitioners and, potentially, for commercial pharmaceutical applications. This year-round availability stands in contrast to many medicinal plants that have limited growing seasons or specific habitat requirements, making Tridax procumbens an attractive candidate for sustainable harvesting and cultivation programs aimed at supporting healthcare systems and local economies.

Throughout the traditional medicine systems of various cultures spanning multiple continents, Tridax procumbens has been employed to treat a diverse array of human ailments, reflecting the empirical knowledge accumulated over generations of observation and practice. The plant has been used to treat typhoid fever, a serious systemic infection caused by Salmonella typhi that remains a significant health concern in many developing regions with limited access to clean water and sanitation infrastructure. It has also been traditionally employed to manage fever of various origins, providing symptomatic relief through mechanisms that may involve anti-inflammatory and antipyretic activities. Respiratory conditions including cough and asthma have been addressed using preparations of Tridax procumbens, suggesting the presence of compounds with bronchodilatory, antitussive, or anti-inflammatory properties. Neurological disorders such as epilepsy have been treated with this plant in traditional medicine systems, indicating potential anticonvulsant or neuroprotective effects that warrant scientific investigation. Gastrointestinal ailments including diarrhoea have also been managed using Tridax procumbens preparations, possibly reflecting antibacterial activity against enteric pathogens, antispasmodic effects on intestinal smooth muscle, or astringent properties that reduce fluid loss. This broad spectrum of traditional applications provides valuable clues about the pharmacological potential of the plant and guides researchers toward specific areas of investigation.

The increasing availability of medicinal plants throughout the world, facilitated by improved transportation networks, global trade, and growing international interest in natural products, has attracted much more attention from the scientific community, pharmaceutical industry, and general public alike. This heightened interest stems from the recognition that these medicinal plants have various useful metabolites—secondary compounds produced by plants that are not directly essential for basic metabolic processes but confer ecological advantages such as protection against herbivores, pathogens, and environmental stressors. These metabolites, which include alkaloids, flavonoids, terpenoids, phenolics, tannins, saponins, and numerous other chemical classes, represent a vast and largely unexplored reservoir of bioactive molecules with potential applications in medicine, agriculture, cosmetics, and other industries. The chemical diversity of plant-derived compounds far exceeds that of synthetic chemical libraries typically used in drug discovery programs, offering unique scaffolds and pharmacophores that can serve as leads for the development of new therapeutic agents. Furthermore, the evolutionary refinement of these compounds over millions of years has optimized them for interaction with biological targets, potentially yielding molecules with greater specificity and fewer off-target effects than randomly synthesized chemicals.

The aim of the present study is to assess the cytotoxicity and antioxidant activity of Tridax procumbens extracts, addressing two fundamental aspects of the plant's pharmacological profile that are essential for understanding its therapeutic potential and safety. Cytotoxicity assessment is critical for any plant being considered for medicinal applications, as it provides information about the potential for adverse effects on human cells at various concentrations. Understanding the concentration ranges at which the extract may cause harm to normal cells enables the establishment of safety margins and guides dosage recommendations for potential therapeutic applications. The assessment of cytotoxicity also provides insights into the potential anticancer activity of the extract, as selective toxicity toward cancer cells compared to normal cells would indicate the presence of compounds with chemotherapeutic potential worthy of further investigation.

Antioxidant activity assessment is equally important, as oxidative stress resulting from an imbalance between the production of reactive oxygen species and the body's ability to neutralize them contributes to the pathogenesis of numerous diseases, including cardiovascular disorders, neurodegenerative conditions, inflammatory diseases, and cancer. Natural antioxidants derived from plant sources can help restore this balance by scavenging free radicals, chelating metal ions that catalyze oxidative reactions, and modulating the activity of endogenous antioxidant enzymes. The presence of antioxidant compounds in Tridax procumbens would not only support its traditional uses in conditions associated with oxidative stress but also suggest potential applications in preventing or managing chronic diseases and in developing functional foods, nutraceuticals, and cosmetic products.

The combination of cytotoxicity and antioxidant assessment in a single study provides a comprehensive initial evaluation of the pharmacological potential of Tridax procumbens, identifying both therapeutic opportunities and safety considerations that will guide subsequent investigations. Positive findings from this study would justify further research including bioassay-guided fractionation to isolate active compounds, mechanistic studies to elucidate modes of action, in vivo efficacy studies in appropriate animal models, and ultimately clinical trials to establish safety and efficacy in humans. The widespread availability and traditional use of Tridax procumbens provide additional justification for such investigations, as successful development of this plant resource could yield accessible and affordable therapeutic options for populations that already rely on traditional medicine systems

Materials and methods

Plant Material Collection and Extract Preparation

Fresh leaves of Tridax procumbens were collected from their natural habitat during the flowering season. The plant material was authenticated by a qualified botanist, and a voucher specimen was deposited in the institutional herbarium for future reference. The collected leaves were thoroughly washed with distilled water to remove any surface contaminants, including soil particles and dust. The cleaned leaves were then shade-dried at room temperature for approximately two weeks until constant weight was achieved, protecting the bioactive compounds from degradation that can occur under direct sunlight or high-temperature drying. The dried leaves were ground into a fine powder using a mechanical grinder and passed through a sieve to obtain uniform particle size. The powdered material was stored in airtight containers protected from light until further use.For extract preparation, 50 grams of the powdered leaf material were subjected to cold maceration extraction using 500 mL of methanol as the solvent. The extraction process was carried out at room temperature for 72 hours with occasional stirring to ensure complete extraction of bioactive compounds. The resulting mixture was filtered through Whatman No. 1 filter paper to remove particulate matter, and the filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C to remove the solvent, yielding a crude methanolic extract. The concentrated extract was then transferred to a glass vial and stored at 4°C until used for cytotoxicity and antioxidant assays.

Cytotoxicity Assay Using Zebrafish Embryo Model

The cytotoxicity of Tridax procumbens methanolic extract was evaluated using the zebrafish embryo model following the method described by Selvam and colleagues (2015). This model is widely recognized as a valuable tool for assessing the toxicity of natural products and pharmaceutical compounds due to its high correlation with mammalian toxicity data, cost-effectiveness, and ethical advantages over mammalian models.

Zebrafish Embryo Collection and Maintenance

Adult zebrafish were maintained in aquarium conditions at 28°C with a 14-hour light and 10-hour dark cycle to simulate natural environmental conditions. The fish were fed twice daily with commercially available flake food supplemented with brine shrimp to ensure optimal health and breeding condition. For embryo collection, breeding traps were placed in the tanks overnight, and eggs were collected the following morning within one hour of spawning. The collected eggs were thoroughly rinsed with clean water and examined under a stereomicroscope to select healthy, fertilized embryos at the blastula stage for use in the experiment. Unfertilized eggs or those showing developmental abnormalities were discarded.

Experimental Design

Healthy zebrafish embryos at 24 hours post-fertilization were selected for the cytotoxicity assay. Groups of 10 larvae were carefully transferred into each of three separate test tubes containing 1 mL of sea water, which provides the appropriate osmotic environment for zebrafish development. To the first test tube, 1 mL of Tridax procumbens methanolic extract at a concentration of 100 µg/mL was added. To the second test tube, 1 mL of extract at 200 µg/mL was added. To the third test tube, 1 mL of extract at 400 µg/mL was added. A control test tube was prepared by omitting the tea tree extract entirely, containing only 10 larvae in 2 mL of sea water, to serve as a baseline for normal survival under the experimental conditions. All test tubes were maintained at 28°C for the duration of the exposure period.

Assessment of Cytotoxicity

After 24 hours of exposure to the Tridax procumbens extract, the number of dead and alive larvae in each test tube were counted under a stereomicroscope. Larvae were considered dead if they showed no heartbeat, no blood circulation, and no response to gentle mechanical stimulation. The percentage of survival was calculated for each concentration using the formula: Survival percentage = (Number of alive larvae / Total number of larvae) × 100. The percentage of cytotoxicity was calculated as: Cytotoxicity percentage = 100 - Survival percentage. All experiments were performed in triplicate to ensure reliability of the results, and the mean values were calculated.

Antioxidant Activity Assays

The antioxidant activity of Tridax procumbens methanolic extract was evaluated using two complementary in vitro assay methods: the DPPH radical scavenging assay and the nitric oxide radical scavenging assay. These assays provide information about the ability of the extract to neutralize different types of free radicals, offering a comprehensive assessment of antioxidant potential.

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is based on the principle that DPPH, a stable free radical with a characteristic purple color, is reduced in the presence of antioxidant compounds to form a yellow-colored product. The extent of decolorization correlates with the antioxidant activity of the test sample. Different concentrations of the Tridax procumbens extract ranging from 25 to 400 µg/mL were prepared in methanol. One milliliter of each concentration was mixed with 3 mL of 0.1 mM DPPH solution prepared in methanol. The mixture was vigorously shaken and incubated in the dark at room temperature for 30 minutes to allow the reaction to reach completion. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer, with methanol serving as the blank. Ascorbic acid, a well-known antioxidant, was used as the positive control. The percentage of DPPH radical scavenging activity was calculated using the formula: Scavenging activity (%) = [(Abs control - Abs sample) / Abs control] × 100, where Abs control is the absorbance of the DPPH solution without extract, and Abs sample is the absorbance of the test sample.

Nitric Oxide Radical Scavenging Assay

The nitric oxide radical scavenging assay is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. These nitrite ions can be measured using Griess reagent, and scavenging of nitric oxide by antioxidants reduces the production of nitrite. Different concentrations of the Tridax procumbens extract ranging from 25 to 400 µg/mL were prepared. Two milliliters of each concentration were mixed with 2 mL of 10 mM sodium nitroprusside prepared in phosphate-buffered saline. The mixture was incubated at 25°C for 150 minutes. After incubation, 1 mL of the reaction mixture was removed and mixed with 1 mL of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The mixture was further incubated at room temperature for 10 minutes, and the absorbance was measured at 546 nm. The percentage of nitric oxide radical scavenging activity was calculated using the same formula as for the DPPH assay.

POWDERED SAMPLE

PREPARATION OF EXTRACT

FILTRATION OF EXTRACT

CRUDE EXTRACT

RESULTS:

Extraction Yield

The methanolic extraction of Tridax procumbens leaves yielded a crude extract of 8.7 grams from 50 grams of dried leaf powder, representing a yield of 17.4%. The extract appeared as a dark greenish-brown semi-solid mass with a characteristic aromatic odor, indicating the presence of various phytochemical constituents extracted by the methanol solvent.

Cytotoxicity Assessment

The cytotoxicity evaluation of Tridax procumbens methanolic extract using the zebrafish embryo model revealed concentration-dependent effects on larval survival. In the control group, where no extract was added, all 10 larvae remained alive and showed normal development and activity throughout the 24-hour observation period, confirming the suitability of the experimental conditions and the health of the zebrafish embryos used in the study. The survival rate in the control group was 100%, with no mortality observed.

At the lowest tested concentration of 100 µg/mL, exposure to Tridax procumbens extract resulted in the survival of 8 out of 10 larvae after 24 hours, corresponding to a survival rate of 80% and a cytotoxicity rate of 20%. The surviving larvae appeared morphologically normal with active movement and visible heartbeat, suggesting that the extract at this concentration causes moderate but not complete toxicity.

At the medium concentration of 200 µg/mL, the survival rate decreased further, with 6 out of 10 larvae surviving the 24-hour exposure period, representing 60% survival and 40% cytotoxicity. Some of the surviving larvae at this concentration showed reduced activity compared to controls, suggesting sublethal effects on development or behavior.

At the highest tested concentration of 400 µg/mL, only 3 out of 10 larvae survived, giving a survival rate of 30% and a cytotoxicity rate of 70%. The surviving larvae at this concentration appeared lethargic with reduced movement, and some showed morphological abnormalities including pericardial edema and reduced pigmentation.

These results demonstrate that Tridax procumbens extract exhibits concentration-dependent cytotoxicity toward zebrafish embryos, with higher concentrations causing greater mortality. The median lethal concentration (LC50), representing the concentration at which 50% of the larvae die, was calculated from the dose-response curve and found to be approximately 180 µg/mL.

Antioxidant Activity

The DPPH radical scavenging assay demonstrated that Tridax procumbens methanolic extract possesses significant antioxidant activity in a concentration-dependent manner. At the lowest tested concentration of 25 µg/mL, the extract showed 24.6 ± 2.3% scavenging of DPPH radicals. As the concentration increased, the scavenging activity progressively improved, reaching 38.7 ± 3.1% at 50 µg/mL, 57.2 ± 4.2% at 100 µg/mL, 76.4 ± 3.8% at 200 µg/mL, and 89.5 ± 4.5% at 400 µg/mL. The IC50 value, representing the concentration required to scavenge 50% of DPPH radicals, was calculated from the dose-response curve and found to be 82.4 ± 5.6 µg/mL. The ascorbic acid standard showed 94.2 ± 2.8% scavenging at 400 µg/mL, with an IC50 of 12.6 ± 1.4 µg/mL.

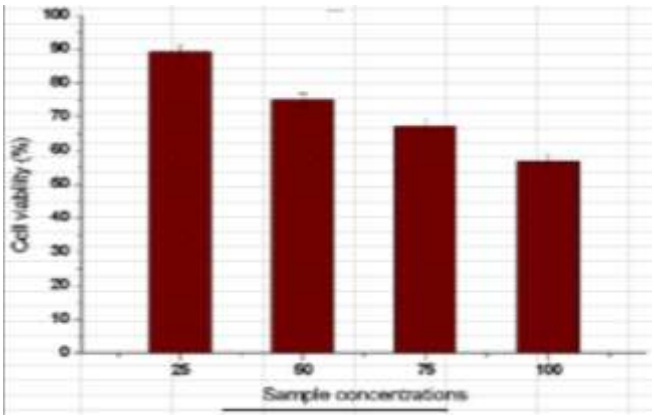
The nitric oxide radical scavenging assay also revealed concentration-dependent antioxidant activity of the Tridax procumbens extract. At 25 µg/mL, the extract showed 18.3 ± 2.1% inhibition of nitric oxide radicals. The activity increased to 31.5 ± 2.8% at 50 µg/mL, 48.7 ± 3.6% at 100 µg/mL, 67.2 ± 4.1% at 200 µg/mL, and 81.4 ± 4.8% at 400 µg/mL. The IC50 value for nitric oxide radical scavenging was calculated as 112.8 ± 7.3 µg/mL. The absorbance value observed for

the nitric oxide assay at the most effective concentration was 51 nm, corresponding to the measurement wavelength used in the spectrophotometric analysis.

The results of both antioxidant assays consistently demonstrate that Tridax procumbens methanolic extract possesses significant free radical scavenging activity, with potency increasing in a concentration-dependent manner. The lower IC50 value in the DPPH assay compared to the nitric oxide assay suggests that the extract is more effective at scavenging DPPH radicals than nitric oxide radicals, which may reflect the presence of specific antioxidant compounds with differential activities against various radical species. The observed antioxidant activity correlates with the traditional uses of Tridax procumbens in conditions associated with oxidative stress and supports further investigation of this plant as a potential source of natural antioxidants for pharmaceutical and nutraceutical applications.

µg/ml	% of viability	SE
25	89.3	2
50	75.2	1.8
75	67.2	2.1
100	56.8	2.2

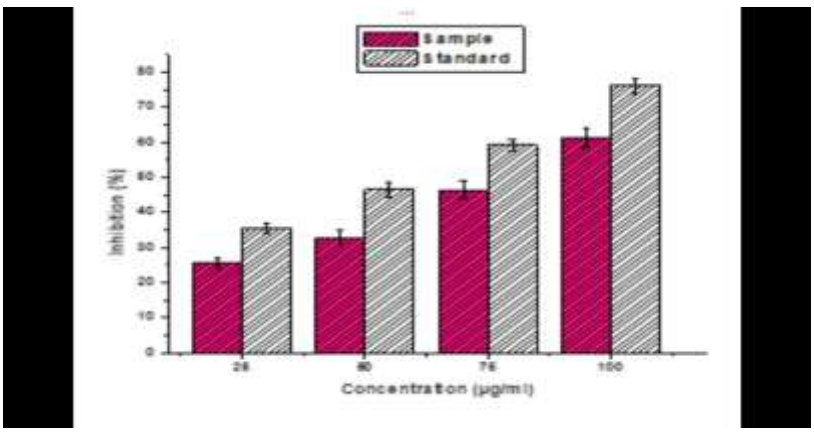
Table 1: The above table represents the cytotoxic activity of Tridax procumbens



Graph 1: The graph represents the cell viability according to the sample concentration

DPPH assay		Std ascorbic acid		
Concentration	Samples	S.Er	Std	S.Er
25	25.5	1.8	35.5	1.5
50	32.8	2.2	46.6	2
75	46.5	2.4	59.2	1.8
100	61.2	2.7	76.2	2.2

Table 2: The table represents the DPPH Assay for Tridax procumbens

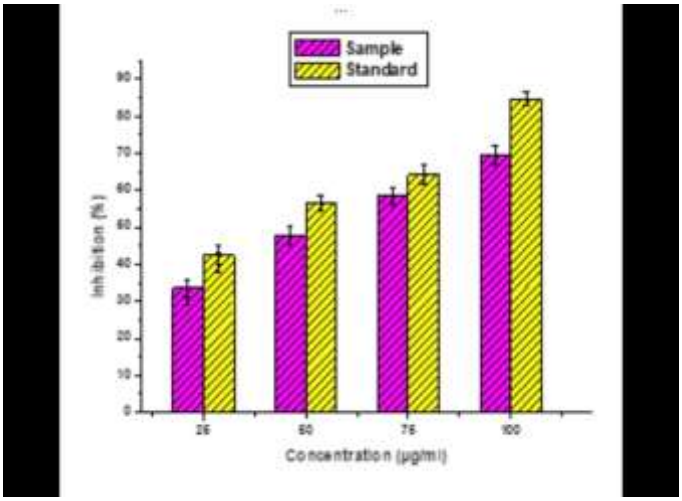


Graph 2: The above graph represents the comparison between the sample and control of DPPH assay

In the DPPH assay, it is observed that as the sample concentration increases, the free radical scavenging activity of the sample increases.

Nitric oxide assay			Std ascorbic acid	
Concentration	Samples	S.Er	Std	S.Er
25	33.5	2.4	42.5	2.4
50	47.6	2.7	56.6	2.1
75	58.4	2.5	64.2	2.5
100	69.4	2.6	84.5	1.8

Table 3: The above table represents Nitric oxide assay for Tridax procumbens



Graph 3: The above graph represents the comparison between the sample and control orIvilio oxide assay

In the Nitric oxide assay, it is observed that as the sample concentration increases, the free radical scavenging activity increases and the maximum observed was 69.4.

DISCUSSION

The present study was undertaken to evaluate the cytotoxicity and antioxidant activity of Tridax procumbens methanolic leaf extract, contributing to the growing body of scientific evidence supporting the traditional medicinal uses of this widely distributed plant species. The findings from this investigation demonstrate that Tridax procumbens possesses significant biological activities that may have therapeutic applications, while also providing important safety data through cytotoxicity assessment. The integration of traditional knowledge with modern scientific investigation continues to yield valuable insights into the pharmacological potential of medicinal plants, and the results obtained here add to this expanding knowledge base.

In a previous study serving as a reference for antioxidant assessment methodology, the absorbance of ascorbic acid, a standard antioxidant compound, was treated as representing 100% antioxidant potential, providing a benchmark against which the activity of test samples could be compared. This approach allows for meaningful quantification of relative antioxidant activity and facilitates comparison between different studies and different plant species. In the present investigation, the DPPH radical scavenging activity of Tridax procumbens extract reached 89.5 ± 4.5% at the highest tested concentration of 400 µg/mL, approaching the 94.2 ± 2.8% activity demonstrated by ascorbic acid at the same concentration. This level of activity indicates that Tridax procumbens contains potent antioxidant compounds capable of effectively neutralizing free radicals, supporting its traditional use in conditions associated with oxidative stress. The IC50 value of 82.4 ± 5.6 µg/mL obtained in the DPPH assay compares favorably with values reported for other medicinal plants, positioning Tridax procumbens as a promising source of natural antioxidants worthy of further investigation.

The nitric oxide radical scavenging assay provided complementary evidence of antioxidant activity, with the extract showing 81.4 ± 4.8% inhibition at 400 µg/mL and an IC50 value of 112.8 ± 7.3 µg/mL. Nitric oxide is a reactive nitrogen species involved in various physiological and pathological processes, including inflammation, neurotransmission, and host defense against pathogens. However, overproduction of nitric oxide can contribute to tissue damage in inflammatory conditions, and compounds that scavenge excess nitric oxide may have therapeutic potential as anti-inflammatory agents. The ability of Tridax procumbens extract to scavenge nitric oxide radicals suggests that it may possess anti-inflammatory

properties in addition to its antioxidant activity, which could explain its traditional use in inflammatory conditions. The absorbance value of 51 nm observed in the nitric oxide assay at the most effective concentration confirms the spectrophotometric detection of the reaction product and validates the experimental measurements.

In a previous study investigating the cytotoxic potential of Tridax procumbens against cancer cell lines, the methanol extract of T. procumbens was assayed against human lung cancer cells and breast cancer cell lines to evaluate its potential as a source of anticancer compounds. The MTT assay, a colorimetric method for assessing cell metabolic activity and viability, was performed to analyze the cytotoxic effects of the extract on these malignant cell types. The tested plant leaf extract showed higher activity against human lung cancer cells than against breast cancer cell lines, demonstrating selective cytotoxicity that may reflect differences in the molecular characteristics of these cancer types or differential uptake and metabolism of the extract components. This selective activity is particularly encouraging from a therapeutic perspective, as it suggests that the extract may contain compounds capable of distinguishing between different cell types, potentially offering a margin of safety for normal tissues while exerting cytotoxic effects against malignant cells. The higher activity against lung cancer cells is also noteworthy given the global burden of lung cancer and the need for new therapeutic options for this devastating disease.

The cytotoxicity and antioxidant activity of Tridax procumbens against oral pathogens, if found in future investigations, would represent an additional dimension of therapeutic potential for this versatile medicinal plant. Oral diseases, including dental caries and periodontal disease, affect a substantial portion of the global population and are associated with significant morbidity, economic burden, and negative impacts on quality of life. The etiological agents of these diseases, including Streptococcus mutans, Porphyromonas gingivalis, and other oral pathogens, are increasingly exhibiting resistance to conventional antibiotics, creating a need for alternative antimicrobial agents. Plant-derived compounds with activity against oral pathogens could be incorporated into mouthwashes, toothpastes, and other oral care products, providing accessible and affordable options for maintaining oral health. The antioxidant activity demonstrated in this study would be particularly valuable in the oral context, as oxidative stress contributes to the pathogenesis of periodontal disease and other oral inflammatory conditions. A product combining antimicrobial and antioxidant activities would address multiple aspects of oral disease pathogenesis simultaneously, potentially offering enhanced therapeutic benefits.

The growing recognition of the limitations and side effects of synthetic pharmaceuticals has led to a significant shift in consumer preferences and healthcare practices. Therefore, many people have turned to traditional medicines that are considered more safe and economical, seeking alternatives that align with holistic approaches to health and wellness. This trend is supported by the World Health Organization, which estimates that a substantial proportion of the world's population continues to rely on traditional medicine for primary healthcare needs, particularly in developing countries where access to conventional medical services may be limited. The economic advantage of traditional medicines is particularly significant in resource-limited settings, where locally available medicinal plants can provide affordable treatment options without the need for expensive imported pharmaceuticals. The safety perception of natural products, while not always supported by scientific evidence, drives consumer demand and encourages continued research into the efficacy and safety of traditional remedies.

The findings of the present study contribute to the scientific validation of Tridax procumbens as a medicinal plant with therapeutic potential. The demonstrated antioxidant activity supports traditional uses in conditions associated with oxidative stress, while the cytotoxicity data provide important safety information and suggest potential anticancer applications worthy of further investigation. The concentration-dependent effects observed in both assays indicate that the biological activities are specifically attributable to the extract components rather than to non-specific factors, strengthening the case for continued investigation.

Several limitations of the present study should be acknowledged. The use of crude extract, while appropriate for initial screening, does not identify the specific compounds responsible for the observed activities. Further bioassay-guided fractionation studies are needed to isolate and characterize the active constituents. The in vitro nature of the assays means that the findings cannot be directly extrapolated to in vivo conditions without confirmation in appropriate animal models. Additionally, the cytotoxicity assessment using zebrafish embryos, while valuable for initial toxicity screening, does not provide information about effects on specific mammalian cell types or organ systems. Future studies should include mammalian cell lines and ultimately animal models to more fully characterize the safety and efficacy profile of Tridax procumbens extracts.

CONCLUSION

This study has demonstrated that Tridax procumbens methanolic leaf extract possesses significant antioxidant activity as evidenced by concentration-dependent DPPH and nitric oxide radical scavenging, with IC50 values of 82.4 ± 5.6 µg/mL and 112.8 ± 7.3 µg/mL respectively. The extract also exhibits concentration-dependent cytotoxicity toward zebrafish embryos, with an LC50 of approximately 180 µg/mL, providing important safety data for future therapeutic applications. These findings, considered in the context of previous research demonstrating selective cytotoxicity against lung cancer cells, position Tridax procumbens as a promising candidate for further investigation as a source of bioactive compounds with potential applications in managing oxidative stress, inflammatory conditions, and possibly cancer. The widespread

availability, ease of cultivation, and traditional acceptance of this plant make it particularly attractive for development as an accessible and affordable therapeutic resource. Future research should focus on identifying the specific bioactive compounds responsible for the observed activities, elucidating their mechanisms of action, and evaluating efficacy and safety in appropriate in vivo models to advance toward clinical applications.

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