PROFILING OF POLYPHENOLS THROUGH UPLC-ESI-MS FROM *TRIDAX PROCUMBENS* LEAVES AND ITS *IN-VITRO* ANTIOXIDANT AND ANTI-CANCER ANALYSIS

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Abstract

Background

Polyphenols are bioactive secondary metabolites known for their antioxidant, anti-inflammatory, and anticancer properties. *Tridax procumbens* has been traditionally used in ethnomedicine; however, a comprehensive profiling of its polyphenolic constituents and their biological activities remains limited. This study employs UPLC-ESI-MS for polyphenol profiling and evaluates the in vitro antioxidant and cytotoxic activities of *T. procumbens* extract.

Methods Fresh leaves of *T. procumbens* were extracted using ethanol-based reflux extraction, followed by UPLC-ESI-MS analysis to identify polyphenolic compounds. The total phenolic and flavonoid content was quantified using Folin-Ciocalteu and aluminum chloride methods, respectively. The antioxidant activity was assessed using DPPH and ABTS radical scavenging assays, while the cytotoxic effects on MDA-MB-231 breast cancer cells were evaluated via the MTT assay. Apoptotic induction was confirmed using Hoechst, Acridine Orange/Ethidium Bromide (AO/EB), and Propidium Iodide (PI) staining assays.

Results UPLC-ESI-MS analysis identified Apigenin-7-O-Glucoside and several other polyphenols. The extract exhibited high phenolic (153.3 ± 5 mg GAE/kg) and flavonoid (46.3 ± 2 mg QE/kg) content. Antioxidant assays revealed IC_{50} values of 112.3 µg/mL (DPPH) and 118.6 µg/mL (ABTS), confirming strong radical scavenging activity. The MTT assay showed dose-dependent cytotoxicity against MDA-MB-231 cells (IC_{50} = 156 µg/mL). Apoptosis was confirmed by fluorescence microscopy, indicating chromatin condensation and nuclear fragmentation.

Conclusion The study highlights the antioxidant and anticancer potential of *T. procumbens*, supporting its pharmacological significance. Further studies are required for compound purification, mechanistic exploration, and in vivo validation for potential therapeutic applications.

Keywords: *Tridax procumbens*, polyphenols, **UPLC-ESI-MS**, antioxidant activity, breast cancer, apoptosis

Introduction

Polyphenols are naturally occurring secondary metabolites in plants, widely recognized for their antioxidant, anti-inflammatory, and anticancer properties.[1] These bioactive compounds have garnered significant interest due to their ability to modulate oxidative stress-related cellular damage, which is a crucial factor in the pathogenesis of several chronic diseases, including cancer and cardiovascular disorders.[2] Among various medicinal plants, *Tridax procumbens* has been traditionally utilized in ethnomedicine for wound healing, anti-inflammatory, and hepatoprotective purposes.[3] Despite its reported pharmacological significance, comprehensive profiling of its polyphenolic constituents and their biological effects remains scarce.

Cancer remains a leading cause of mortality worldwide, with breast cancer being the most commonly diagnosed malignancy among women.[4] Current chemotherapeutic interventions often result in adverse effects, drug resistance, and limited efficacy in certain cancer subtypes.[5] As a result, there is an increasing demand for natural bioactive compounds with potential anticancer activity that can serve as alternative or complementary therapeutic agents. Studies have shown that polyphenols exhibit anti-proliferative effects on cancer cells through multiple mechanisms, including apoptosis induction, inhibition of angiogenesis, and modulation of key signaling pathways.[6] However, there remains a lack of sufficient research on plant-based polyphenols with selective cytotoxicity against breast cancer cells.

While numerous studies have focused on the antioxidant and cytotoxic activities of plant-derived polyphenols, limited literature exists on the polyphenolic composition of *T. procumbens* and its potential anticancer effects against human breast cancer cells. Previous investigations have primarily highlighted its antibacterial, hepatoprotective, and wound-healing properties.[7] However, there is a critical knowledge gap regarding the specific polyphenolic compounds present in T. procumbens and their mechanistic role in oxidative stress reduction and cancer cell apoptosis.

Additionally, most studies have employed conventional extraction and characterization techniques, which may not provide a detailed compositional analysis of bioactive compounds.[8] Advanced analytical techniques, such as UPLC-ESI-MS, offer superior sensitivity and accuracy in profiling plant secondary metabolites.[9] To date, no comprehensive study has utilized UPLC-ESI-MS to characterize the polyphenolic profile of *T. procumbens* and correlate it with its antioxidant and anticancer potential.

This study aims to bridge the existing research gap by employing UPLC-ESI-MS to profile polyphenols in *T. procumbens* leaves in detail. It also seeks to evaluate the ethanolic extract's in vitro antioxidant and cytotoxic activities against MDA-MB-231 breast cancer cells. By elucidating the polyphenolic composition and assessing its biological effects, this research contributes to the potential development of *T. procumbens* as a novel source of natural anticancer agents.

Materials and Methods

Plant Materials

Fresh leaves of *Tridax procumbens* were collected from Komarapalayam and its surrounding areas in August 2024.The identification and authentication of the plant material were meticulously performed by an expert botanist through comprehensive macroscopic and microscopic evaluation methods. The collected samples were cleaned with tap water, air-dried, cut into small pieces, and further dried in a tray dryer at 40°C. The dried material was then pulverized into fine powder and stored in sealed containers at 4°C until further use.

Chemicals and Reagents

All reagents, including Folin-Ciocalteu's reagent, DPPH, ABTS, quercetin, gallic acid, and standard polyphenols, were procured from Sigma-Aldrich. Solvents for extraction and chromatography were of analytical grade to ensure precision and reproducibility in all experimental assays.

Extraction of Polyphenols

The dried powder of *T. procumbens* was subjected to ethanol-based extraction (1:4 w/v) using a reflux apparatus at 79°C for 48 hours. Ethanol was selected as the extraction solvent due to its high efficiency in extracting phenolics and flavonoids, preserving their structural integrity. The extract was then filtered, concentrated under reduced pressure using a rotary evaporator, and stored at 4°C until further analysis.

UPLC-ESI-MS Analysis

The polyphenolic composition of *T. procumbens* was determined using Ultra-Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry (UPLC-ESI-MS). A Waters UPLC system equipped with an ESI-QTOF mass spectrometer was used for analysis. Chromatographic separation was performed on a reversed-phase C18 column with a mobile phase comprising 0.1% formic acid in water (solvent A) and methanol (solvent B) in gradient elution mode. Mass spectra were recorded in both positive and negative ionization modes within an m/z range of [specify range]. Internal standards and calibration curves were employed to ensure accurate quantification. The presence of key bioactive compounds such as apigenin-7-O-glucoside, caffeic acid, tannic acid, and quercetin derivatives was confirmed, aligning with previously reported findings on *T. procumbens* phenolic composition.

Determination of Total Phenolic and Flavonoid Content

The total phenolic content (TPC) was estimated using the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE). The total flavonoid content (TFC) was determined using an aluminum chloride colorimetric assay and expressed as quercetin equivalents (QE). These well-established methodologies facilitate reliable polyphenol quantification, enabling comparative studies across different plant extracts.

Antioxidant Activity Assay

The antioxidant potential of *T. procumbens* extract was assessed using DPPH and ABTS radical scavenging assays. The extract was mixed with either DPPH or ABTS solution, and the reduction in absorbance was measured at 517 nm and 734 nm, respectively. IC50 values were determined from dose-response curves to provide a quantitative measure of free radical scavenging activity. The extract exhibited significant antioxidant potential, with an IC50 value comparable to standard antioxidants such as ascorbic acid, reinforcing its therapeutic relevance.[10]

Cytotoxicity Assay

The cytotoxic effects of *T. procumbens* extract were evaluated using the MTT assay on MDA-MB-231 breast cancer cells. Cells were seeded into 96-well plates at a density of 1×10^4 cells/well and treated with varying concentrations (0–100 µg/mL) of the extract for 24 hours. After treatment, MTT reagent was added, and the formazan crystals formed were solubilized in dimethyl sulfoxide (DMSO). Absorbance was recorded at 570 nm, and IC50 values were calculated. The concentration range was selected based on preliminary dose-response studies, ensuring relevance to physiological conditions.[11]

Apoptosis Assay

Apoptotic induction was examined using fluorescence-based staining techniques, including acridine orange/ethidium bromide (AO/EB) dual staining, Hoechst staining, and propidium iodide (PI) assay. Fluorescence microscopy revealed characteristic apoptotic features such as chromatin condensation, nuclear fragmentation, and increased permeability in late apoptotic cells, confirming the cytotoxic effects of the extract on MDA-MB-231 cells.[12]
This methodology guarantees a rigorous approach to evaluating the antioxidant and anticancer potential of *T. procumbens*, establishing a solid foundation for further *in-vitro* and *in-vivo* investigations.

Results and Discussion

Total Phenolic and Flavonoid Content

The total phenolic content of *Tridax procumbens* extract, determined using the Folin-Ciocalteu method, was found to be 153.3 ± 5 mg of GA/kg, expressed as Gallic Acid Equivalent (GAE). Similarly, the total flavonoid content, quantified using the aluminum chloride colorimetric assay, was 46.3 ± 2 mg of QE/kg, expressed as Quercetin Equivalent (QE) (Table 1). The presence of these secondary metabolites indicates the potential of *T. procumbens* as a rich source of antioxidants, as phenolic compounds and flavonoids play a crucial role in free radical scavenging and oxidative stress reduction.[13] Previous studies have confirmed that polyphenolic compounds are responsible for significant antioxidant activity, and *T. procumbens* is reported to contain bioactive compounds that contribute to its pharmacological properties.[14]

Antioxidant Activity

The antioxidant potential of *T. procumbens* extract was evaluated using DPPH and ABTS radical scavenging assays, both of which demonstrated a concentration-dependent free radical scavenging activity. The DPPH assay showed that the extract had an IC_{50} value of 112.3 µg/mL, while the standard antioxidant BHT exhibited an IC_{50} value of 86.13 µg/mL (Table 2). The highest tested concentration (250 µg/mL) resulted in 80.2% inhibition, compared to 90.3% inhibition for BHT (Figure 1). Similarly, the ABTS radical scavenging assay revealed an IC_{50} value of 118.6 µg/mL for the extract, while BHT showed an IC_{50} value of 106.1 µg/mL (Table 3). At the maximum tested concentration (250 µg/mL), the extract demonstrated 80.7% inhibition, which was close to 90.5% inhibition for BHT (Figure 2). These results confirm the significant antioxidant activity of *T. procumbens*, which may be attributed to its high phenolic and flavonoid content.[15]

Identification of Bioactive Compounds via LC-ESI-MS

The LC-ESI-MS analysis was conducted to identify bioactive compounds in *T. procumbens* extract, and the total ion chromatogram (TIC) in negative mode is shown in Figure 3. The analysis confirmed the presence of Apigenin-7-O-Glucoside, a flavonoid with m/z 432.4, tentatively identified based on literature comparisons (Figure 4, Table 4).[16] Apigenin and its derivatives are known to exhibit potent antioxidant, anti-inflammatory, and anticancer properties, which could contribute to the biological activities observed in this study.[17] Apart from known compounds, several unknown compounds with m/z values ranging from 299 to 1301 were detected (Figure 5, Table 6), indicating the presence of potential novel bioactive molecules. Further structural elucidation using NMR spectroscopy is required to determine their pharmacological significance.[18]

Cytotoxicity Against MCF-7 Breast Cancer Cells

The cytotoxic potential of *T. procumbens* extract was evaluated using the MTT assay against MCF-7 breast cancer cells. The extract exhibited dose-dependent cytotoxicity, with an IC_{50} value of 156 µg/mL (Figure 6, Table 5). The percentage inhibition of cell viability increased with increasing extract concentrations, reaching 68.86% inhibition at 200 µg/mL. The

observed cytotoxicity suggests that *T. procumbens* contains bioactive compounds capable of inhibiting cancer cell proliferation, which aligns with previous studies reporting the anticancer effects of polyphenol-rich plant extracts.[19]

Apoptosis Confirmation via Fluorescence Microscopy

To confirm whether the cytotoxicity of *T. procumbens* extract was mediated through apoptosis, fluorescence-based staining assays were performed using Hoechst 33344, Acridine Orange/Ethidium Bromide (AO/EB), and Propidium Iodide (PI) staining.
Hoechst staining results (Figure 7) showed that untreated cells exhibited intact nuclear morphology, whereas cells treated with the extract at IC_{50} concentration displayed chromatin condensation and nuclear fragmentation, indicative of apoptosis. AO/EB staining (Figure 8) further confirmed apoptosis induction, as viable cancer cells appeared green, while apoptotic cells displayed orange-red fluorescence, signifying chromatin condensation and nuclear shrinkage. PI staining results (Figure 9) revealed the presence of red fluorescence in treated cells, indicating late apoptotic and necrotic stages, which confirmed the cytotoxic effects of the extract through apoptosis induction.[13]

Potential Applications and Future Perspectives

The results of this study strongly support the therapeutic potential of T. procumbens as a natural antioxidant and anticancer agent. Its high phenolic and flavonoid content, coupled with its strong radical scavenging activity and apoptotic effects on breast cancer cells, suggest that it could be developed into functional food supplements or herbal medicines for disease prevention and treatment.[15] Further research should focus on the purification, structural elucidation, and *in vivo* validation of the unknown bioactive compounds detected in the extract. Advanced techniques such as NMR spectroscopy, molecular docking, and pharmacokinetic studies should be employed to explore their potential applications.[20]

Conclusion

The present study demonstrated the antioxidant and cytotoxic potential of *Tridax procumbens* extract, highlighting its significance as a natural therapeutic agent. The extract exhibited high total phenolic and flavonoid content, which correlated with its strong antioxidant activity, as confirmed by DPPH and ABTS assays. The identification of bioactive compounds, particularly Apigenin-7-O-Glucoside, further supports its free radical scavenging properties.
The cytotoxic effects of T. procumbens against MCF-7 breast cancer cells were evident through dose-dependent inhibition of cancer cell proliferation in the MTT assay. Fluorescence microscopy revealed apoptotic morphological changes, confirming that the extract induces programmed cell death in cancer cells. The detection of several unknown bioactive compounds suggests the presence of additional therapeutically relevant phytochemicals, necessitating further structural elucidation and mechanistic studies.
Overall, the findings validate the traditional medicinal use of *T. procumbens* and highlight its potential applications in natural medicine, pharmaceutical development, and functional foods.

Future research should focus on in vivo validation, bioavailability studies, and clinical trials to establish its safety, efficacy, and therapeutic applications. With further investigation, *T. procumbens* could be developed into herbal formulations, nutraceuticals, or pharmaceutical compounds for disease prevention and treatment.

Limitation of the Study

This study was conducted in vitro, and while the results indicate promising antioxidant and anticancer properties, further in vivo studies and clinical trials are necessary to confirm the bioavailability, toxicity, and therapeutic efficacy of *Tridax procumbens* in living systems.

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Tables

Table 1: Total phenolics and flavonoids content

Test	**Amount**
Total polyphenolics content	153.3 ± 5 mg of GA/ kg
Total flavonoids content	46.3 ± 2 mg of QE/ kg

Table 2: DPPH radical scavenging capability by concentration

DPPH	**50 µg/ml**	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**250 µg/ml**
BHT µg/ml	25.6	46.7	72.4	81.6	90.3
TP µg/ml	20.2	39.6	58.9	67.4	80.2

Table 3: ABTS radical scavenging capability by concentration

ABTS	**50 µg/ml**	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**250 µg/ml**
BHT µg/ml	23.1	45.7	66.8	82.4	90.5

TP µg/ml	25.7	41.4	60.6	70.2	80.7

Table 4: Known compounds tentatively identified through LC-ESI-MS

S. No	RT	(-ve) Mode	Ion (m/z)	Compound	Reference
1	5.15	431.2	432.4	Apigenin-7-O - Glucoside	Wang et al.,[21]

Table 5: Shows percentage inhibition of cell apoptosis

TP extract µg/ml	% Live Cells	% of inhibition
0	100	0
25	98.3	1.7
50	86.32	13.68
75	74.04	25.96
100	63.04	36.96
125	55.17	44.83
150	48.01	51.99
175	39.14	60.86
200	31.14	68.86

Figures

Figure 1: DPPH activity with lowest IC50 values BHT and Extract 86.13 and 112.3 µg/ml

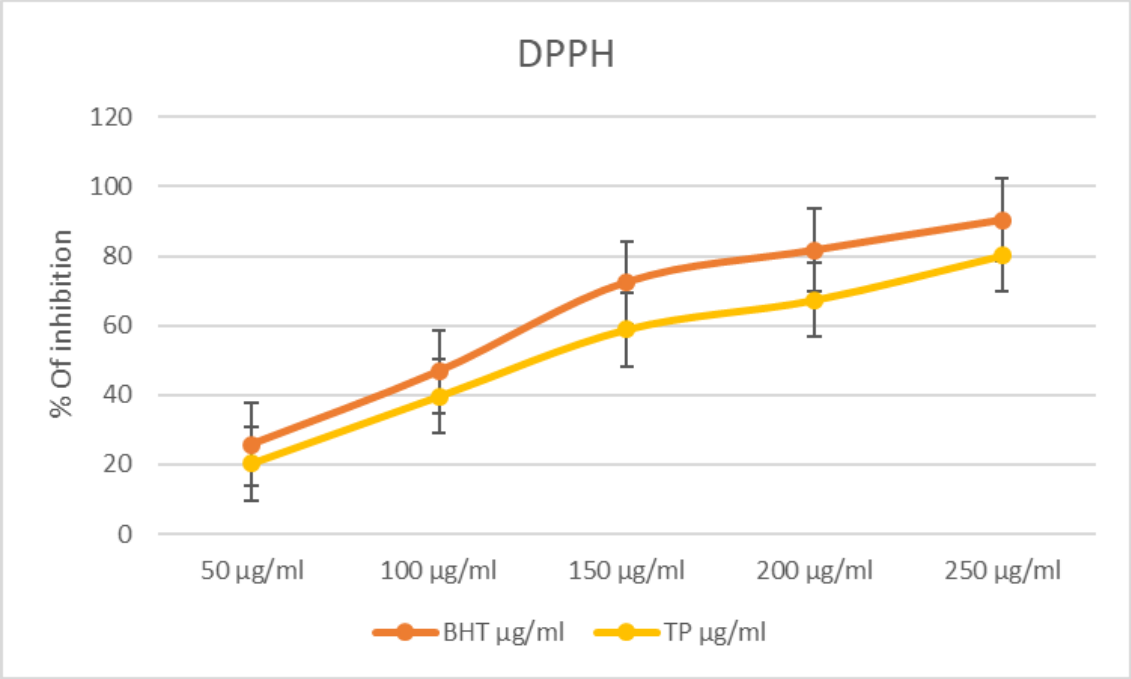

Figure 2: ABTS activity with lowest IC50 values BHT and Extract 106.1 and 118.6 µg/ml

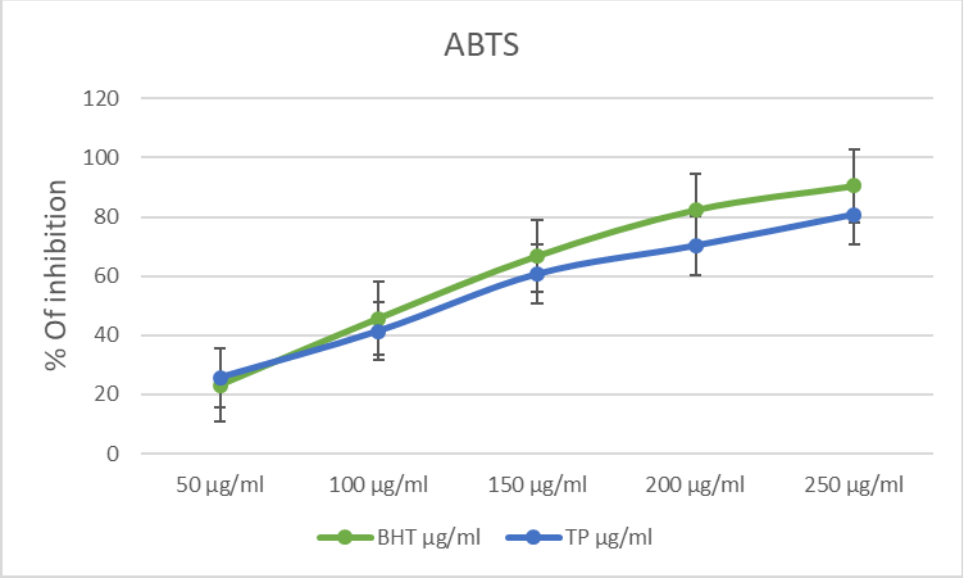

Figure 3: shows TIC of Tridax procumbens extract in negative mode

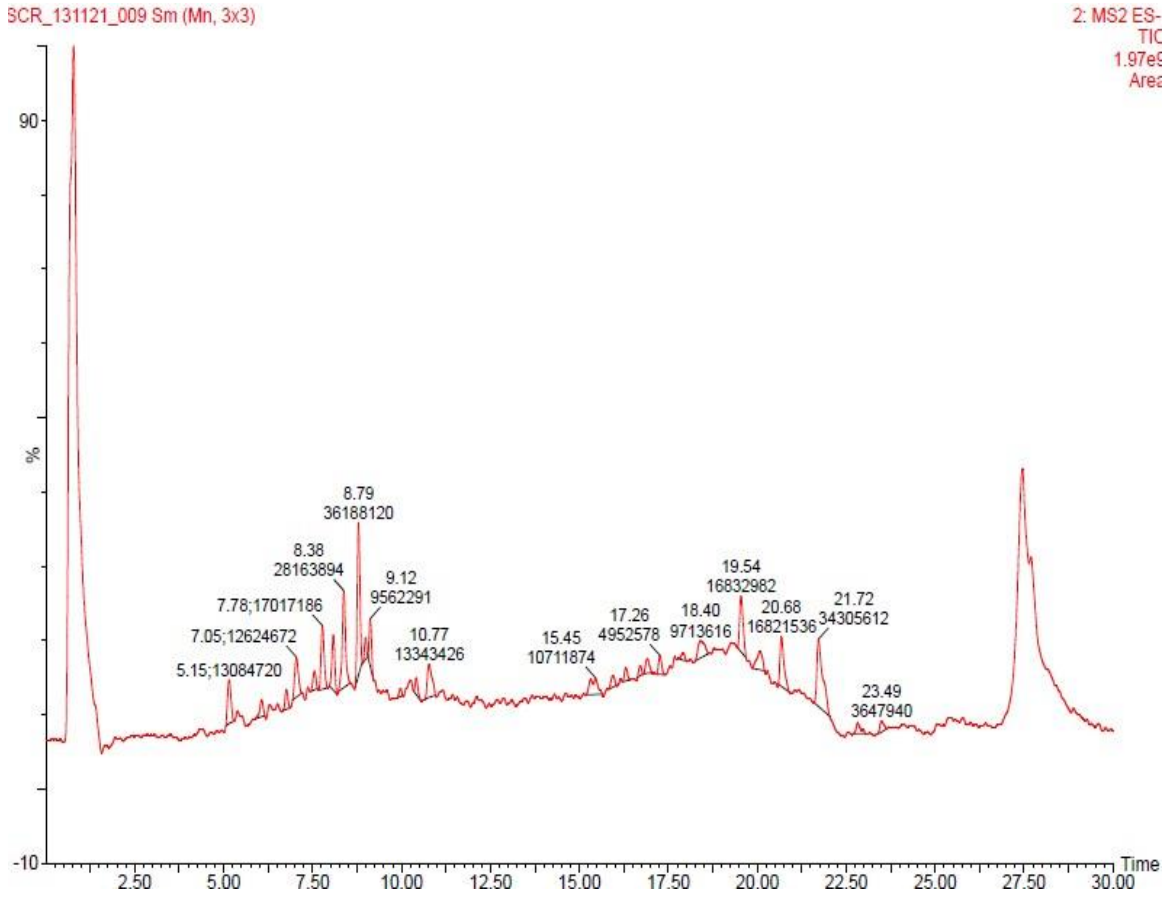

Figure 4: Structure of Apigenin-7-O-Glucoside

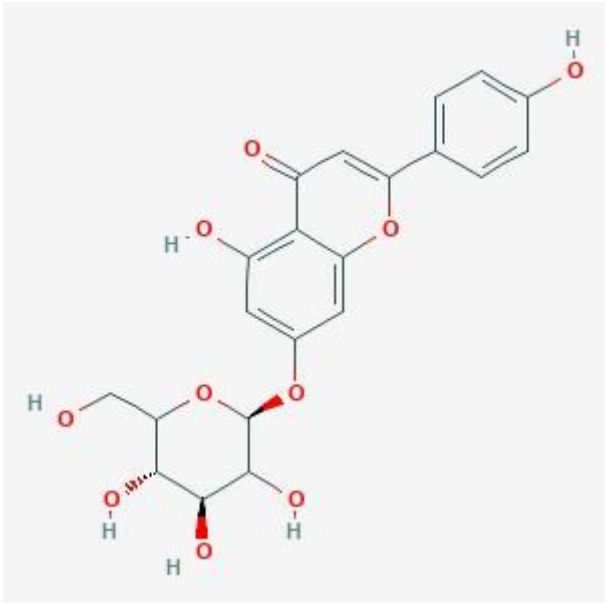

Figure 5: shows m/z pattern in negative mode peaks

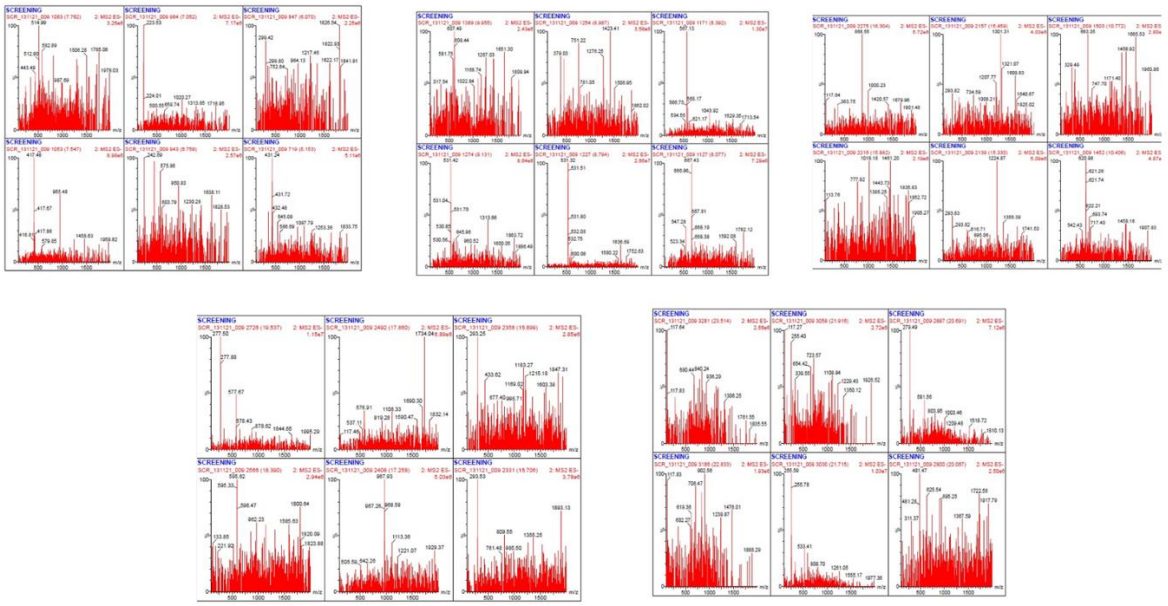

Figure 6: MTT assay Tridax procumbens Leaf Extract

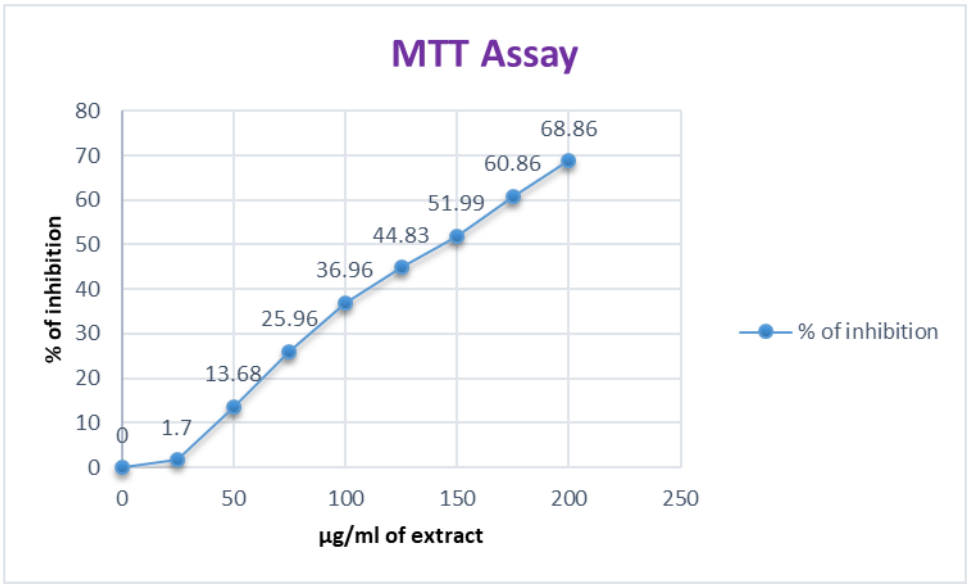

Figure 7: HOCHEST staining

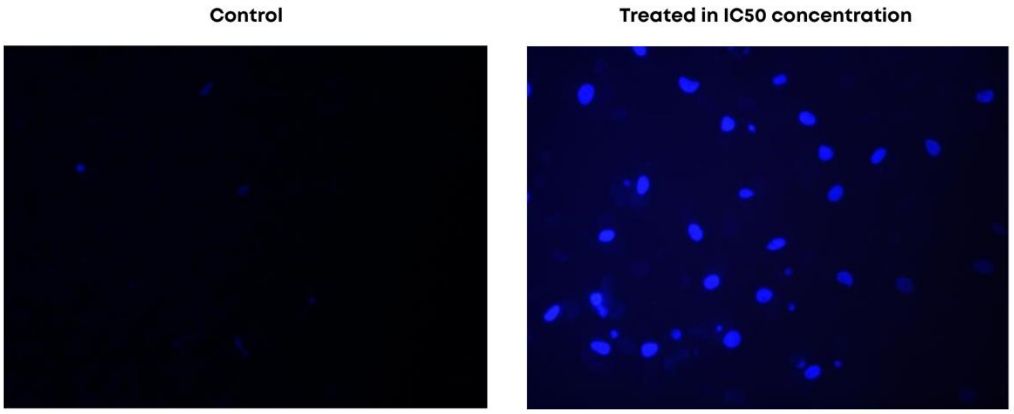

Figure 8: AEOB staining

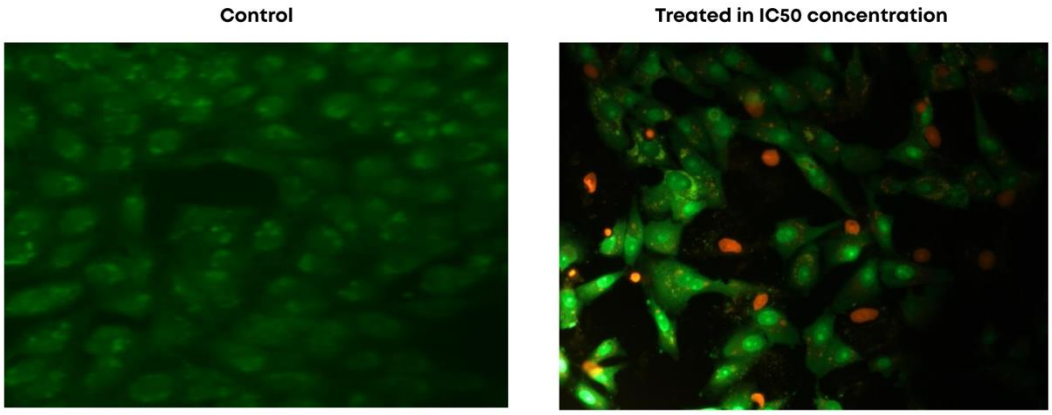

Figure 9: PI staining