A study on the anti-cancer, anti-oxidant and [1]anti-bacterial activities of Alternanthera ficoidea (L.) R. Br against human breast cancer cells (MCF-7)

M Hemanth Kumar[1], Saravana P[2], Bharath Kumar K[3], Anshu Beulah Ram[4]
[1,2,3] *Department of PG Studies in Biotechnology, Nrupathunga University, Nrupathunga Road, Bengaluru 567 001, Karnataka, India*
[4]*Faculty, Department of PG studies in Biotechnology, Nrupathunga University, Nrupathunga Road, Bangalore 560 001, Karnataka, India.*

***Abstract*—Background: Breast cancer is a major cause of cancer-related deaths worldwide. It could be caused by various factors like increasing age, obesity, harmful use of alcohol etc. Objectives : This research aimed to investigate the antioxidant, antibacterial, and anticancer activities of Alternanthera ficoidea (L.) R. Br. Methods : Different parts of the plant extract were prepared, DPPH assay for antioxidant activity, Disc diffusion assay for antibacterial activity and MTT assay for anticancer activity were determined using standard assay protocol. Cell cycle studies and GC-MS analysis were performed on leaf extract. Result: Leaf extract showed significant activity as antioxidant activity (IC50=130.2µg/mL), antibacterial activity (20 ± 0.6 mm) against Staphylococcus aureus and Escherichia coli shows negative results and anticancer activity (IC50 = 61.36µg/mL). Cell cycle studies showed arrest of cells at "G0-G1" and "S"-phase. GC-MS results showed key components with antioxidant, anticancer compounds, and anti-inflammatory activities. Conclusion: Plant based medicines are safer and reliable. Given these promising results, Alternanthera ficoidea presents a valuable opportunity for developing novel plant-based drugs for cancer treatment.**

***Index Terms*—Alternanthera ficoidea; Anti-cancer activity; MTT assay; GC-MS; Cytotoxicity; Anti-oxidant activity; Anti-bacterial activity; Flow cytometry.**

I. INTRODUCTION

Cancer is the second leading cause of death globally, accounting for an estimated around 3,10,720 new cases of invasive breast cancer will be diagnosed in women in United states only, in 2024 [1]. It is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body [2]. Breast cancer is one of the most dangerous and leading causes of cancer-related deaths. In 2022, there were 2.3 million women diagnosed with breast cancer and 670,000 deaths globally [3]. Chemotherapy is one of the widely used treatments for various cancer types. However, their effectiveness is limited and often causes adverse effects. Hence finding safe and reliable medications from natural sources is hot topic of interest.

Plants have abundant secondary metabolites with potential activities against cancer, microbes and free radicals. Plants were used as herbal medicines for their potential properties. Alternanthera ficoidea (family Amaranthaceae) also known as "Joseph's coat" is a glabrescent herb (Figure 1), native to South America. The leaves are lance late, oblanceolate, or linear-oblong in shape, measuring around 5 cm in length, sometimes reaching up to 10 cm, and about 1 cm in breadth, occasionally extending to 2 cm. The inflorescence consists of 1-3 sessile axillary heads. The perianth is subequal, glabrous, 2 mm long, and 1-nerved. There are three stamens present. Flowering occurs throughout the year, and each plant produces one seed [4]. Alternanthera ficoidea is a traditional herbal plant known for their diuretic, anti-pyretic, antiseptic, anti-inflammatory and anti-diarrheal properties [5]. The leaves are eaten raw with rice, boiled as a potherb, or eaten with Sambal, a hot pepper mixture [6]. Alternanthera ficoidea plant extracts have been reported to have anti-microbial, anti-

inflammatory, anti-cancer and hepatoprotective properties.

Various types of phytochemicals are present in Alternanthera genera with wide spectrum of biological activities such as diuretic, anti-pyretic, antiseptic, anti-inflammatory, anti-diarrheal anti-oxidant, anti-cancer, antimicrobial and anti-inflammatory activities [7]. Hence, this study aims to explore the activities of A. ficoidea leaf, stem and root extracts. This work may aid in featuring the potential therapeutic approach of A. ficoidea plant.

II. MATERIALS AND METHODS

Plant Materials : The whole A. ficoidea. plant was collected from the fields of Malur taluk, Kolar district, Karnataka, India. The plant was authenticated by the Central Institute of Ayurveda Research Institute, Bengaluru. The plant samples were shade dried at room temperature for a week and then in hot air oven for a day at 60˚C.

Preparation of Extracts: The shade - dried leaves, stem and root samples were ground with a mechanical grinder to obtain fine powder and extracted by using methanol as a solvent. The extraction was carried out in water bath for 4 hours at 64˚C. After 4hours of extraction the sample was filtered using Whatmann No.1 filter paper. The extract obtained were subjected to evaporation until methanol traces evaporated.

Phytochemical Analysis: The qualitative analysis for the presence of phytochemical compounds were performed according to Shaik., et al. 2020 [8]. The methanolic extracts of leaf, stem and roots were used for phytochemical screening.

Anti-oxidant Assay : 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried out as per the method of Rajakumar et al. (49). In brief, 80µL of DPPH solution with various concentration of test solution was made sufficient to 240µl with HPLC grade methanol [10]. The different concentrations tested for reference standard were 0.156, 0.3125, 0.625, 1.25, 2.5, 5µg/mL. The concentrations tested for test samples were 6.25, 12.5, 25, 50, 100, 200µg/mL [11]. The reaction mixture was mixed and incubated at 25°C for 15 minutes. The absorbance was measured at 510 nm using spectrophotometry. A control reaction was also included without the test sample [12].

Cytotoxicity assay using MTT

Cell culture: MCF-7 cell lines were procured from American Type Culture Collection (ATCC) stock cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% inactivated Fetal Bovine Serum (FBS), penicillin (60µg/mL), streptomycin (100µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent [13]. The cells were dissociated using 0.05% trypsin and centrifuged at 1000 rpm for 5 minutes. The culture media was discarded and the cell pellet was gently re-suspended using 2 ml DMEM complete media [14]. The viability of the cells was checked and a single cell suspension of 5.0 x 10^5 cells/mL was prepared [15].

Treatment: To each well of the pre-labelled 96-well microtiter plate, 100 µL of the prepared cell suspension (50,000 cells/well) was added and incubated at 37°C with 5% CO_2. After 24 hours of incubation, the supernatant was removed and the monolayer was rinsed with DMEM. To each pre-designated well, 100µL of test drugs at various concentrations were added and incubated for 24hours. After incubation, the test solutions in the wells were discarded and 100 µL of MTT reagent 4 mg/10 mL of MTT in Phosphate buffer saline (PBS) was added to each well. The plates were incubated for 4 h at 37°C in 5% CO_2. The supernatant was removed and 100 µL of Dimethyl sulfoxide (DMSO) was added and the plates were gently shaken to solubilize the formazan crystals. The absorbance was measured using a microplate reader at 590 nm wavelength using a multimode plate reader, Spectra max i3X, Molecular devices. The percentage growth inhibition was calculated using the following formula and the concentration of test drug to inhibit cell growth by 50% (IC50) values is generated from the dose-response curves for each cell line using GraphPad Prism 5.0 software [16][17][18][19].

Cell cycle studies using Flow Cytometry: Cells were plated at a density of 1 x 10^6 cells per well in a 6-well plate in DMEM cell culture medium and incubated under standard conditions of 5% CO_2 at 37°C. After overnight incubation, the medium was gently removed using a pipette and replaced with fresh culture medium (without FBS). Subsequently, cells were treated with respective concentrations of samples and incubated for 24 hours in a CO_2 incubator. The cell culture medium was then collected into ria tubes to prevent cell loss due to cell death [20].

Next, the remaining cells in the 6-well plate were detached using 0.05% trypsin. To stop trypsin activity, 1 ml of medium was added to each well, and the contents were transferred to the Ria tubes. The cell suspension was centrifuged at 4000 rpm for 5 minutes at 4°C, and the supernatant was discarded. Cells were fixed by resuspending them in 300 µl of sheath fluid. Then, 1 ml of chilled 70% ethanol was added drop by drop with continuous gentle shaking, followed by another 1 ml of chilled 70% ethanol added at once. The cells were stored overnight at 4°C [21].

After fixation, the cells were centrifuged again at 4000 rpm for 5 minutes. The cell pellet was washed twice with 2 ml of cold 1X PBS. The cell pellet was then resuspended in 300 µl of sheath fluid containing 0.05 mg/mL propidium iodide (PI) and 0.05 mg/mL RNaseA, and incubated for 15 minutes in the dark. The percentage of cells in various stages of the cell cycle in both treated and untreated populations was determined using a FACS Caliber flow cytometer (BD Biosciences, San Jose, CA) [22]. Following staining, the cells were acquired by the flow cytometer as soon as possible (within 1 hour) using the FL2 filter, and further analyzed using CellQuest software [23].

Anti-bacterial activity:

Cell suspensions of test organisms, adjusted to a cell density of 1-2 x 10^5 cells/mL, were inoculated (100 µL per plate) onto Tryptic soya agar medium (90 mm). Using the spread plate technique the inoculum was uniformly distributed with a sterile glass L-spreader. Agar wells (5 mm) were created on the inoculated agar plates using a sterile cork borer and numbered on the backside of the plates. Test samples (20 µL from 100 mg/mL) were taken from stocks, while standard ciprofloxacin (20 µL from 0.1 mg/mL) and methanol as negative controls (20 µL) were added to the pre-marked wells on the agar plates. The plates with test samples were kept at 2-8°C in a refrigerator for 20 minutes to allow diffusion from the wells and were subsequently incubated at 37°C for 16-24 hours. After incubation, the plates were observed for zones of inhibition, and the results were recorded by calculating the diameter of the clear zone around the wells in mm [24][25].

GC-MS analysis:

The analysis was conducted using Clarus 680 GC with a fused silica column, packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df). The components were separated using helium as the carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. A 1 µL extract sample was injected into the instrument. The oven temperature program was as follows: 60°C for 2 minutes, then increased to 300°C at a rate of 10°C per minute, and held at 300°C for 6 minutes.

The mass detector conditions were: transfer line temperature of 240°C, ion source temperature of 240°C, and ionization mode by electron impact at 70 eV. The scan time was 0.2 seconds with a scan interval of 0.1 seconds, and fragments from 40 to 600 Da were analyzed. The spectra of the components were compared with the database of known components stored in the GC-MS NIST (2008) library [26].

III. RESULTS

Phytochemical Analysis

The methanolic extracts of leaf, stem and root of Alternanthera ficoidea were screened for phytochemicals using standard protocols. As shown in table 1, the leaf extract showed positive results for alkaloids, glycosides, phenols, saponins, tannins and terpenoids. The stem extract showed positive results for alkaloids, glycosides, steroids, saponins and terpenoids. The root sample showed positive results for alkaloids, anthraquinones, carbohydrates, glycosides, phenols, steroids, saponins, tannins and terpenoids.

Anti-oxidant Assay

DPPH radical scavenging activity of leaf, stem and root extract was determined by calculating the percentage inhibition of the free radical. Half maximal Inhibitory concentration (IC50) was calculated using Graph pad Prism 5.0 software. The IC50 values of leaf, stem and root were found to be 130.2µg/mL, 246µg/mL, and 252.3µg/mL respectively. The IC50 value of the standard (Gallic Acid) was found to be 2.169µg/mL (Figure 2). The percentage inhibition was calculated using the below formula :

$$\% \text{ inhibition} = \frac{(\text{OD of control} - \text{OD of sample}) \times 100}{\text{OD of control}}$$

Cytotoxicity assay using MTT:

Cytotoxic assay for leaf, stem and root extracts were performed using MTT assay. Half maximal Inhibitory concentration (IC50) was calculated using Graph pad Prism 5.0 software. The IC50 values of leaf, stem and

root were found to be 61.36µg/mL, 240.7µg/mL and 232.2µg/mL respectively. The IC 50 value of the standard Doxorubicin was found to be 11.47µg/mL. The cytotoxic effects of A. ficoidea on the MCF-7cell line were observed under an inverted compound microscope. At a higher concentration of 640 µg/mL, doxorubicin induced the formation of formazan crystals, indicating cell death, while at a lower concentration of 80 µg/mL, no formazan crystals were observed. Similarly, the leaf, stem, and root extracts also produced formazan crystals at the higher concentration (640 µg/mL), but at the lower concentration (80 µg/mL), no crystals were formed, suggesting reduced cytotoxic activity (Figure 3).

Cell cycle studies using Flow Cytometry

The leaf extract showed a better IC50 value of 61.36 µg/mL as compared to stem and root extract in the cytotoxic assay, the leaf extract was selected for cell cycle studies using the flow cytometric method. At the concentration of 50µg/mL and 100µg/mL, cell cycle arrest was shown to be 13.26% and 29.53% of cells gated in the S phase respectively compared to the control cells having shown 6.77% of cells gated in the same phase. While on other hand 5.77%, 18.65% cell arrest was observed at G_2M phase, respectively (table 2 & figure 5 & 6).

Anti-bacterial activity

The results of the antibacterial activity (table 3) revealed that while S. aureus was sensitive to the leaf and stem extracts of A. ficoidea (figure 7A), no activity was exhibited by the root extract (figure 7B). The maximum zone of inhibition achieved with leaf methanol extract of A. ficoidea for S. aureus was 20± 0.6mm. The extracts failed to show any kind of inhibition against E. coli at the tested concentrations as indicated by (table 3 and figures 7).

GC-MS analysis

The Gas Chromatography /Mass Spectroscopy results for A. ficoidea are shown in Table 4. The GC/MS Analysis revealed the presence of seven bioactive compounds including Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-, (1α,2β,5α)-; 7-tetradecenal (Z); 4-Tridecanol; 22,23-dibromostigmasterol acetate; Ergost-5-en-3-ol, acetate (3β,24R)-; Stigmasta-5,22-dien-3-ol, acetate, (3β)- and Stigmasteryl tosylate (figure 8)

IV. DISCUSSION

This study aimed at evaluating the antioxidant, antibacterial and cytotoxic potentials of different parts of A. ficoidea. The methanolic leaf extract was found to present profound cytotoxic potential as compared to stem and root with IC50 of 61.36 µg/mL, while IC50 of compared standard (Doxorubicin) remained to be 11.47 µg/mL. The IC50 values of stem remained at 240.7µg/mL and root remained at 232.2µg/mL. On the other hand, while study the antibacterial potential of A. ficoidea. was tested against S. aureus and E. coli using leaf, stem, and root extracts. The leaf extract showed strong activity, with an inhibitory zone of 20 ± 0.6 mm at a concentration of 2 mg/well against Staphylococcus aureus, but it was ineffective against E. coli. Similarly, the stem extract demonstrated an inhibitory zone of 18 ± 0.0 mm against S. aureus but showed no significant effect on E. coli. In contrast, the root extract did not exhibit any antibacterial activity. For comparison, the standard antibiotic ciprofloxacin produced inhibition zones of 16.6 ± 0.6 mm at a much lower concentration of 0.002 mg/well against S. aureus and 13.6 ± 0.6 mm against E. coli. While determining the antioxidant potential of A. ficoidea. Leaf showed significant antioxidant potential with IC50 of 130.2µg/mL followed by stem with IC50 of 246µg/mL and root with IC50 value of 252.3µg/mL, when compared to standard (Gallic acid) with IC50 value of 2.169µg/mL. The GC/MS analysis of the leaf of A. ficoidea. revealed the presence of seven bioactive compounds that may contribute to its potential cytotoxic effects. Among these, 7-tetradecenal (Z), 22,23-dibromostigmasterol acetate, and Stigmasta-5,22-dien-3-ol, acetate (3β), were Stigmasta-5,22-dien-3-ol, acetate (3β) binds to the receptor tyrosine kinase domain of EGFR, effectively inhibiting its function. This inhibition is significant because EGFR overexpression enhances progesterone receptor (PR) activity, which promotes breast cancer progression. By targeting both EGFR and PR, this bioactive compound disrupts their interaction, thereby inhibiting downstream signaling pathways and suppressing the growth and proliferation of MCF-7 breast cancer cells [35]. In addition, 22,23-dibromostigmasterol acetate shows promise in the treatment of ovarian cancer, although further studies are needed to confirm its efficacy [32]. Other compounds, such as 4-Tridecanol, Stigmasta-5,22-

dien-3-ol, acetate (3β), and Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-, (1α,2β,5α)-, exhibit antioxidant and anticancer activities [29] [35] [36]. Additionally, Stigmasteryl tosylate demonstrates antibacterial properties, particularly against E. coli and S. aureus [37]. This analysis was further progressed for cell cycle studies by fluorescence-associated cell sorting method using flow cytometry. The cell populations at different stages of the cell cycle treated with different concentrations of leaf extract determined 50µg/mL and 100µg/mL of the drug to be effective in arresting the cell cycle at the 'G0/G1' phase and 'S' phase. Though A. ficoidea is native to Brazil Australia and India for its various uses by locals, this plant is not much focused by the scientific community. Hence, this can serve as source for bringing Novel plant-based drugs for many therapeutics especially in cancer research.

V. ACKNOWLEDGEMENT

The technical support provided by Skanda life sciences private limited, Bangalore, Karnataka, India is highly appreciated.

REFERENCES

[1] American Cancer Society. (n.d.). How common is breast cancer? https://www.cancer.org/cancer/types/breast-cancer/about/how-common-is-breast-cancer.html

[2] National Cancer Institute. (n.d.). What is cancer? National Cancer Institute. https://www.cancer.gov/about-cancer/understanding/what-is-cancer

[3] Union for International Cancer Control. (n.d.). Breast cancer. https://www.uicc.org/what-we-do/thematic-areas/breast-cancer

[4] Kashyap, P. V., Shrikanth, P., & Shetty, N. (2021). Morphological comparison of three different Alternanthera species found in Udupi. International Journal of Botany Studies, 6(2), 582-584. https://www.botanyjournals.com/assets/archives/2021/vol6issue2/6-2-102-742.pdf

[5] Ameer, F., Ali, M. Y., & Shahid, M. (2020). Alternanthera ficoidea: A review of its phytochemistry, pharmacological activities, and traditional uses. International Journal of Pharmaceutical Research, 12(1), 58-66. http://www.ijpronline.com/ViewArticleDetail.aspx?ID=10892

[6] Fruits, T. (n.d.). Alternanthera ficoidea. Tropical Plants Database. Retrieved August 11, 2024, from https://tropical.theferns.info/viewtropical.php?id=Alternanthera+ficoidea

[7] Singla RK, Dhir V, Madaan R, Kumar D, Singh Bola S, Bansal M, Kumar S, Dubey AK, Singla S, Shen B. The Genus Alternanthera: Phytochemical and Ethnopharmacological Perspectives. Front Pharmacol. 2022 Apr 11; 13:769111. doi: 10.3389/fphar.2022.769111. PMID: 35479320; PMCID: PMC9036189.

[8] Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies, 8(2), 603-608.

[9] Rajakumar DV. (1994) Biochemical & Pharmacological studies on the antioxidant properties of Dehydrozingerone and its analogs, university of Mangalore (unpublished Ph. Dthesis).

[10] Burits M, Bucar F. (2000) Phytotherapy Res. 14: 323-328.

[11] Arjun HB, Yasuhiro T, Ketut AI, Kiyoshi M, Katsumichi M, Dejair M, Alfredo AGH, Shigetoshi K. (2000) J. Ethnopharmacol. 72: 239-246.

[12] Deepak, T., Shashank, A. S., Kumar, K. Y., & Rajalakshmi, V. K. S. (2020). Phytochemical analysis, antioxidant and antihemolytic activity of Hibiscus and Amla. International Journal of Science and Research (IJSR), 9(4).

[13] Crouch, S.P.M. et al. (1993). The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J. Immunol. Meth.**160**, 81–8.

[14] Gonzalez, R.J. and Tarloff, J.B. (2001) Evaluation of hepatic sub cellular Fractions for alamar blue and MTT reductase activity. Toxicology in vitro.15, 259-9.

[15] Hattori, N. et al. (2003) Enhanced microbial biomass assay using mutant luciferase resistant to benzalkonium chloride. Anal. Biochem.319287–95.

[16] Kangas, L. et al. (1984) Bioluminescence of cellular ATP: A new method for Evaluating cytotoxic agents in vitro. Med. Biol. 62, 338–43.

[17] Landin, A. et al. (1986) Estimation of biomass in growing cell lines by adenosine triphosphate assay, Methods enzymol.133,27-42.

[18] Cell Viability and Proliferation. Mark Frei, BioFiles v6 n5, 17–21.

[19] F FDenizot, R R Lang. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunological Methods 1986-05-22.

[20] Joany Jackman and Patrick M. O'Connor. Methods for Synchronizing Cells at Specific Stages of the Cell Cycle. Current Protocols in Cell Biology.1998, 8.3.1-8.3.20.

[21] Vajihe Azimian-Zavareh, Ghamartaj Hossein, Ehsan Janzamin. Effect of lithium chloride and anti-neoplastic drugs on survival and cell cycle of androgen-dependent prostate cancer LNCap cells. Indian Journal of Pharmacology. 2012 Dec, Vol 44, Issue 6, 714-721.

[22] Ramesh P. Thylur, Senthivinayagam S, Campbell EM, Rangasamy V, Thorenoor N, Sondarva G, Mehrotra S, Mishra P, Zook E, Le PT, Rana A, Rana B. Mixed Lineage Kinase 3 Modulates β-Catenin Signaling in Cancer Cells. J. +-Biol Chem. 2011 Oct 28; 286(43):37470-82.

[23] Kasibhatla, S., Amarante-Mendes, G. P., Finucane, D., Bronner, T., Bossy-Wetzel, E., & Green, D. R. (2006). Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. Cold Spring Harbor Protocols, 2006(3), pdb-prot449.

[24] Barry, A. L., and L. D. Sab h. 1974. Special tests: bactericidal activity and activity of antimicrobics in combination, p. 431-435. InIE. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.

[25] Bergeron, M. G., J. L Brusch, M. Barza, and L. Weinstein. 1973. Bactericidal activity and pharmacology of cefazolin. Antimicrobe. Agents Chemother.4:396-401.

[26] Manual for GC-MS analysis from VIT-SIF lab, SAS, VIT University

[27] Kavitha Alli and Lakshmi Narasu Mangamoori (2013), comparative evaluation of antimicrobial activities of root, stem and leaves of Holoptelea integrifolia against pathogenic bacteria, Asian Jr. of Microbial. Biotech. Env. Sc. Vol. 16, No. (1): 2014: 145-154.

[28] Godwin Ndarake Enin A, et al., (2024), In vitro Antioxidant and Biological Activities of Extract and Fractions from Telfairia occidentalis Stems, South Asian Research Journal of Natural Products Volume 7, Issue 2, Page 102-122, 2024; Article no. SARJNP.116748.

[29] Yahaya, Gavamukulya et al., (2014), anti-cancer potential of ethanolic and water leaves extracts of Annona muricata (graviola), Jomo Kenyatta university of agriculture and technology, http://hdl.handle.net/123456789/4010.

[30] Dahiru M, et al., (2021), Anogeissus leiocarpus: A Potential Inhibitor of Some Extended Spectrum β-Lactamase Bacteria, Acta Scientific Microbiology (ISSN: 2581-3226) Volume 4 Issue 11 November 2021.

[31] Samina KY, et al., (2019), GC-MS Analysis of Chemical Constituents and in vitro Antioxidant Activity of the Organic Extracts from the Stem of Bridelia stipularis, Sains Malaysiana 48(5) (2019): 999–1009, http://dx.doi.org/10.17576/jsm-2019-4805-08.

[32] Kanika Verma, Shanthi Veerappapillai, et al., (2016); EXPLORATION OF PLANT BIOACTIVE FROM CASSIA FISTULA LEAVES FOR THE TREATMENT OF OVARIAN CANCER: AN INTEGRATIVE APPROACH, Asian Journal of Pharmaceutical and Clinical Research; Vol 9, Issue 5, 2016.

[33] Anusiya G, Bharathi S, et al., (2020); Extraction and molecular characterization of biological compounds from water hyacinth, Journal of Medicinal Plants Studies 2020; 8(5): 14-19, DOI: https://doi.org/10.22271/plants.2020.v8.i5a.1189 .

[34] Sunita Arora and Ganesh Kumar, et al., (2017); Gas Chromatography-Mass Spectrometry (GC-MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of Cenchrus setigerus Vahl (Poaceae); The Pharma Innovation Journal 2017; 6(11): 635-640.

[35] Naik, Bindu,et al., (2019), Phytochemical Analysis of Diplazium esculentum Reveals the Presence of Medically Important Components, Bentham Science Publishers, DOI**:** https://doi.org/10.2174/1573401316999200614162834.

[36] Vartika Mishra 1, Sarika Tomar 2, Singh 1, et al., (2022), Elemental Analysis, Phytochemical Screening and Evaluation of Antioxidant, Antibacterial and Anticancer Activity of Pleurotus ostreatus through In Vitro and In Silico Approaches, Metabolites 2022, 12, 821. https://doi.org/10.3390/metabo12090821

[37] M. Krishnaveni, C. Ragina Banu2, M. Kalaivani2 and G. Krishnakumari2, et al., (2015), GC-MS/MS analysis of phytochemicals in Muntingia calabura l, antimicrobial assay. World Journal of Pharmaceutical Research SJIF Impact Factor 5.045 Volume 4, Issue 4, 1855-1859. Research Article ISSN 2277– 7105.

Tables

Table 1 : Phytochemical screening of *Alternanthera ficoidea*

Name of the compound	Phytochemical test	*Alternanthera ficoidea*		
		Leaf sample	Stem sample	Root sample
Alkaloids	Dragendoff test	+	+	+
Anthraquinone	Bontrager's test	-	-	+
Carbohydrates	Molisch test	-	-	+
Flavonoids	Alkaline reagent test	-	-	-
Glycosides	Conc.H_2SO_4 test	+	-	+
	Killer-Killiani test	+	+	+
Phenols	Ferric chloride test	+	-	+
Steroids	Liebermann-Burchard test	-	+	+
Saponins	Frothing test	+	+	+
Tannins	Braymer test	+	-	+
Terpenoids	Salkowski test	+	+	+

Note: (+) Present; (-) Absent

Table 2 : FACS analysis of Cell cycle arrest in MCF-7 cells, untreated, treated with doxorubicin and treated with leaf extract at two different concentrations.

Cell line	Samples	Flow cytometry analysis of Cell cycle arrest in MCF-7cells				
		Conc. µg/mL	Sub G0	G0/G1	S	G2M
MCF-7	Control	-	10.99	78.97	6.77	6.57
	Doxorubicin	10	0.8	53.22	28.41	18.37
	Leaf extract (S1)	50	6.89	76.54	13.26	5.77
		100	0.8	51.9	29.53	18.65

Table 3: Antibacterial activity of leaf, stem and root extract of Alternanthera ficoidea against Staphylococcus aureus and Escherichia coli.

Zone of Inhibition (mm)			
Alternanthera ficoidea		Bacterial species	
Samples	Concentration per well (mg/well)	Staphylococcus aureus	Escherichia coli
Menthol	20µl	NA	NA
Ciprofloxacin	0.002	16.6± 0.6	13.6± 0.6
Leaf extract	2	20± 0.6	NA
Stem extract	2	18± 0	NA
Root extract	2	NA	NA

Table 4 : GC/MS analysis of *Alternanthera ficoidea* – leaf extract.

S. No	Compound name	Mw (g/mol)	Formula	Rt (min)	Area %	Activities	Ref.
1	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-, (1α,2β,5α)-	138.2	$C_{10}H_{18}$	19.240	52.765	Antibacterial, antioxidant	[27] [28]
2	7-tetradecenal, (z)	210.3	$C_{14}H_{26}O$	19.810	9.392	Anticancer, antimicrobial	[29] [30]
3	4-tridecanol	200.3	$C_{13}H_{28}O$	20.526	1.713	Antioxidant	[31]
4	22,23-dibromostigmasterol acetate	614.5	$C_{31}H_{50}Br_2O_2$	24.972	2.721	Anticancer, antimicrobial, antioxidant	[32] [33]
5	Ergost-5-en-3-ol, acetate, (3β,24r)-	442.7	$C_{30}H_{50}O_2$	25.373	9.288	Anti-inflammatory	[34]
6	Stigmasta-5,22-dien-3-ol, acetate, (3β)-	454.7	$C_{31}H_{50}O_2$	25.553	2.512	Anticancer, Antimicrobial, antioxidants	[35] [36]
7	Stigmasteryl tosylate	566.9	$C_{36}H_{54}O_3S$	25.843	4.120	Anti-microbial	[37]

(10) Figures

Figure 1: (a) *A. ficoidea* whole plant and (b) Leaves

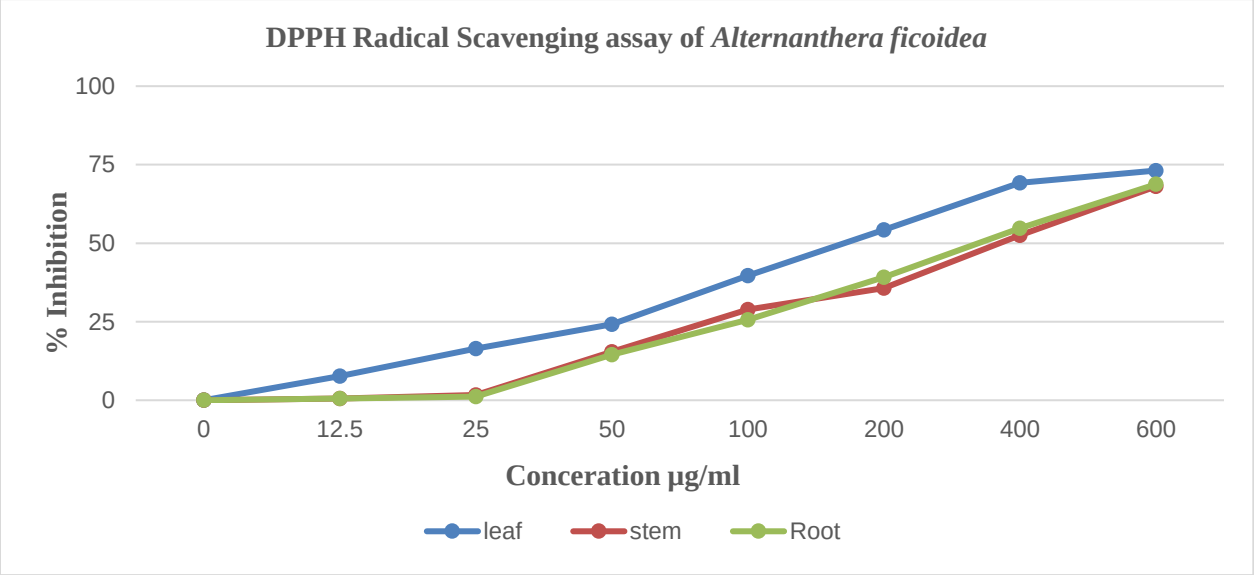


Figure 2: Anti-oxidant assay of leaf (blue line), stem (red line) and root (green line) extracts of Alternanthera ficoidea

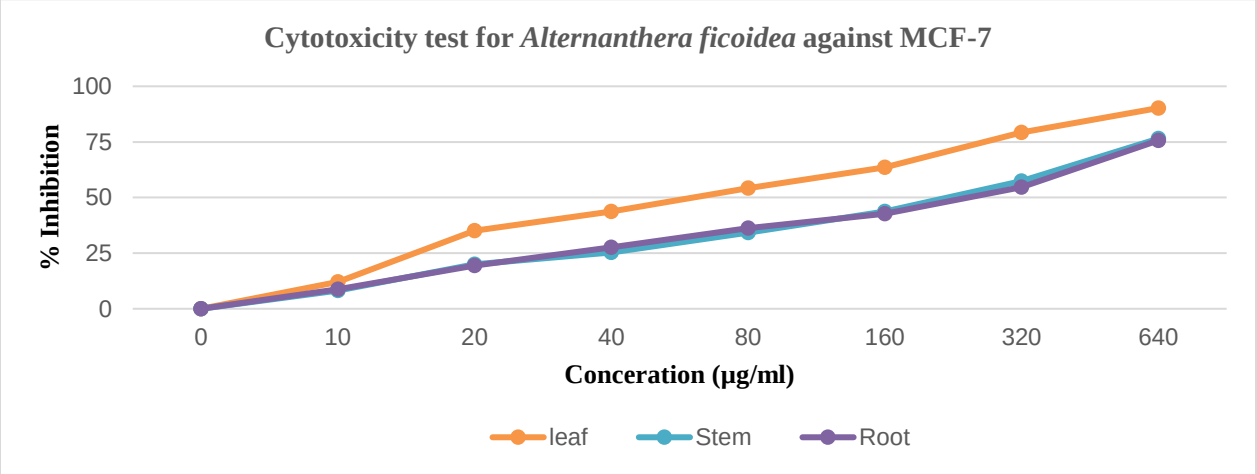


Figure 3: MTT assay using leaf (orange line), stem (blue line) and root (purple line) extracts of A. ficoidea against MCF-7 cell line

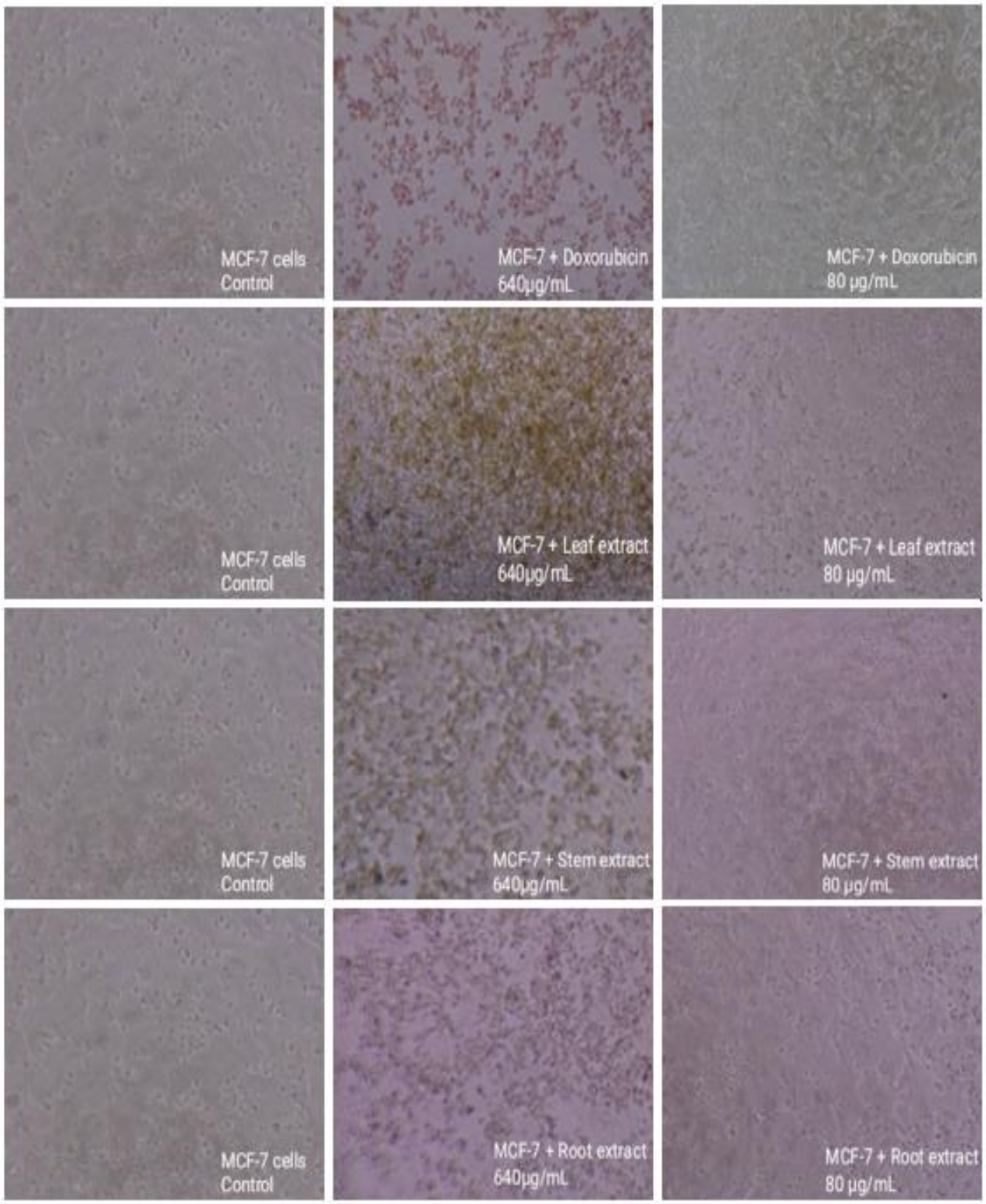


Figure 4: Cytotoxic effects of A.ficoidea on MCF-7 human breast cancer cell line, observed under inverted compound microscope. C – Control; DH – Doxorubicin 640µg/mL; DL – Doxorubicin 80µg/mL; LH – leaf 640µg/mL; LL – leaf 80 µg/mL; SH – stem 640µg/mL; SL – stem 80µg/mL; RH–root640µg/mL; RL–root 80µg/mL.

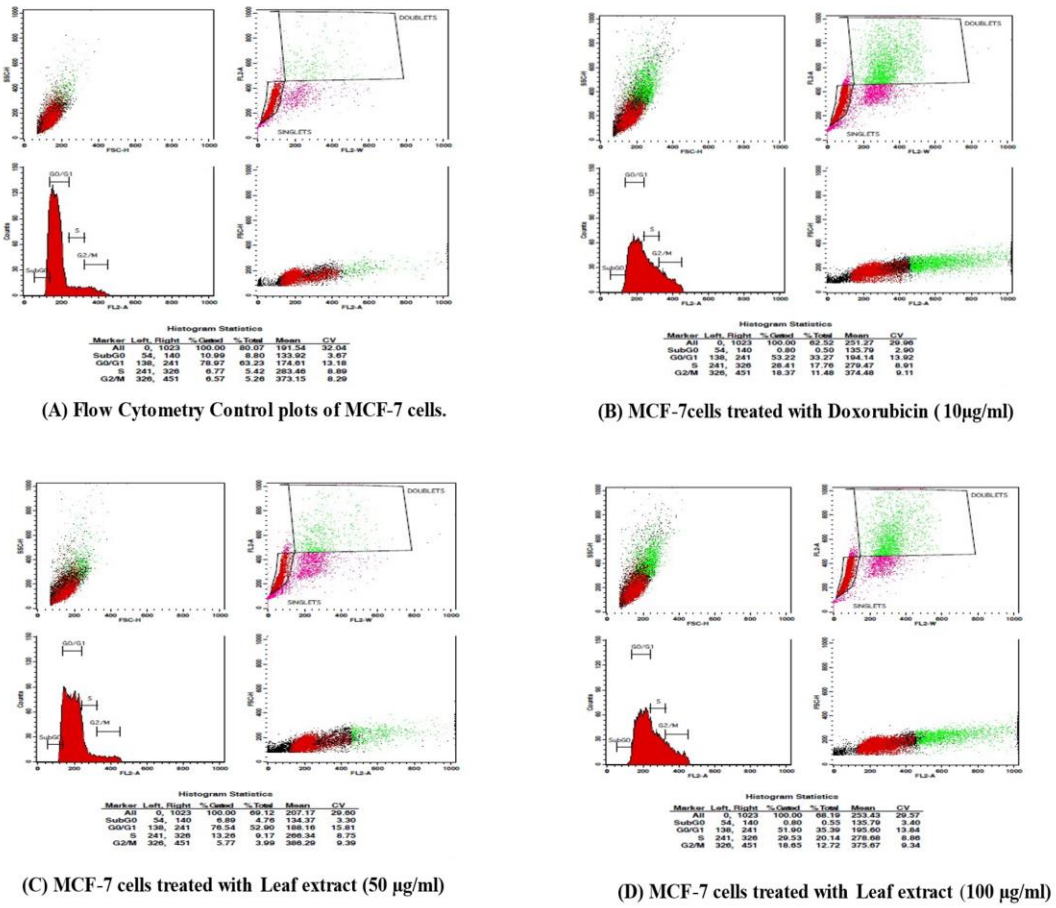


Figure 5: Flow cytometry evaluation of MCF-7 cells, (A) Flow Cytometry Control plots of MCF-7 cells, (B) MCF-7cells treated with Doxorubicin 10µg/mL, (C) MCF-7 cells treated with 50 µg/mL of the sample - Leaf extract, (D) MCF-7 cells treated with 100 µg/mL of the sample - Leaf extract.

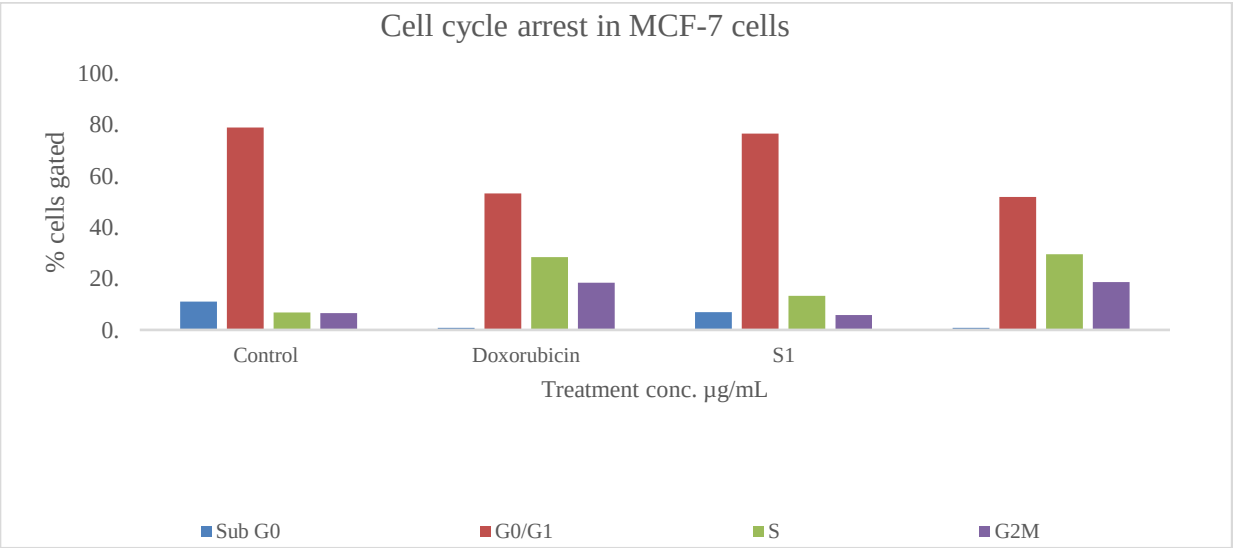
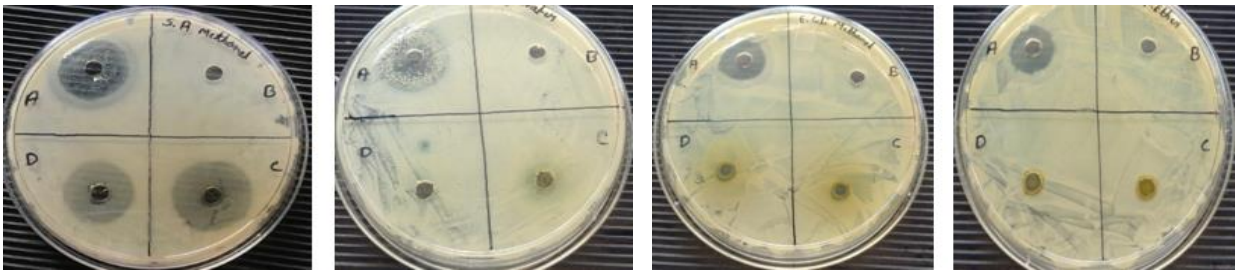


Figure 6: FACS analysis of Cell cycle arrest in MCF-7 cells:



(A) (a) (b) (B) (a) (b)

Figure 7: (A) (a) Staphylococcus aureus: A- Standard (Ciprofloxacin; 0.002 µg/well), B- Control (Methanol), C-Sample (leaf), D- Sample (stem). (b) D- Root
(B) (a) Escherichia coli: A- Standard (Ciprofloxacin; 0.002 µg/well), B- Control (Methanol), C-Sample (Leaf), and D- Sample (Stem). (b) D- Root.

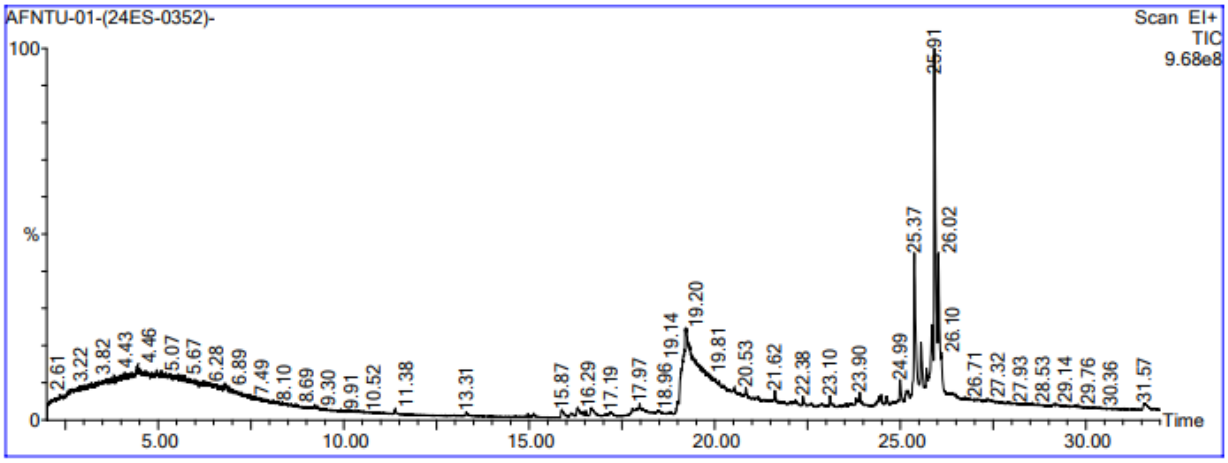


Figure 8: Chromatogram of GC/MS analysis of A.ficoidea – Leaf.

Author's Contribution:

1. M Hemanth Kumar: Designed the study, carried out the experiments, interpreted the results, prepared the manuscript
2. Saravana P: Designed the study, carried out the experiments, interpretated the results, prepared the manuscript
3. Bharath Kumar K: Designed the study, carried out the experiments, interpretated the results, prepared the manuscript
4. Anshu Beulah Ram: Guided the students, corrected and finalized the manuscript.

Financial support and sponsorship: Nil

Conflicts of Interest: There is no conflict of interest