**RESEARCH ARTICLE**

# Isolation, Identification and Screening of the *In vitro* Cytotoxicity of the Phytochemicals from *Duranta erecta* (Verbenaceae)

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**ABSTRACT**

The main objective of the present study was to investigate cytotoxic activity of the phytoconstituent of Duranta *erecta* L. against the HL-60 cell line using SRB assay. Column chromatographic method & Preparative Thin Layer Chromatography was used for the separation of phytochemicals from chloroform extract of the stem of *Duranta erecta***.** Three isolated compounds were identified as well as confirmed by using different analytical techniques like UV, FTIR, $^{1}H$ & $^{13}C$ NMR and MS. *In vitro* cytotoxic activity of these compounds was screened using SRB assay against Leukemia cancer cell lines in humans (HL-60). The compounds Ursolic acid, Pyrocatechol and Kaempferol were separated and confirmed from the chloroform extract. All extracted compounds exhibited inhibition of the HL-60 cell lines and showed $IC_{50}$ values less than 165µg/ml. Moreover, Ursolic acid ($IC_{50}$98.6µg/ml) was more cytotoxic than other isolated compounds when tested on the cell line model.The statistically significant difference ($P<0.05$) in cytotoxic activity of extract was observed relative to standard compound when examined by independent student t test. Amongst three compounds Ursolic acid anticancer activity was different by statistic ($P<0.05$) than Pyrocatechol as well as Kaempferol. Additionally, the compounds which were isolated from the extract of chloroform of D. *erecta* show dose-dependent cytotoxic activity on HL-60 cell lines. This study suggested that the *Duranta erecta* is a promising source of bioactive chemicals having anticancer activity which could be used in the cancer drug discovery process.

**Keywords**: *Duranta erecta;* Cytotoxic activity; Chloroform extract; SRB assay; Human leukemia cancer cell line

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**Conflict of interest:** None

## INTRODUCTION

In 21st century, cancer is a major publically health, societal and economical problem, responsible for one in six deaths worldwide. Cancer is serious disease in which cells expand quickly, uncontrollably, and pathologically as a result of disturbances in normal cell division. Cancer has the ability to spread throughout the body and more than 100 differnt types of cancer have been documented.Several organs in the body, including the lungs, stomach, liver, prostate, bone marrow, lymph nodes, and breasts, are especially susceptible to the formation of secondary cancers when metastasis occurs[1,2]. As Cancer is the main reason of death in the world resposible for estimated 10 million deaths annually. Cancer of lungs was the most subsequently detected cancer in the year 2022, responsible for 2.5M newer cases of cancer world wide ( 12.4% of all the cancer worldwide), and cancers like prostate (7.3%), stomach (4.9%) female breast (11.6%), colorectum (9.6%). Cancer of lung was considered as a main reason of death by cancer, with an estimated death (18.7%)[2].

Currently, the most used cancer therapies are radiation therapy, chemotherapy & surgery, but specific treatments have grown in prominence. Consequently, contemporary efforts to treat cancer concentrate primarily on chemotherapeutic medications, biological products, and immune-mediated therapies[3].

Many existing chemotherapy treatments show inadequate efficacy and significant toxicity, consequently there is need for creative, effective, and non-toxic treatment and medications for cancer therapy. Traditional medicine is used widely across the globe due to its availability, inexpensive, widespread use, and accessibility. While the effectiveness of traditional medicines in the treatment of cancer is thoroughly demonstrated, there is an absence of literature which integrates traditional knowledge and current herbal medicine practice[4-7]. *Duranta erecta* Linn., having family Verbenaceae, is generaly used in Nigeria, the Philippines, Bangladesh, India and Brazil as traditional medicine. *D. erecta* shows variety of biological effects, including antimalarial, antibacterial, antioxidant, cytotoxic, antinephritic, hepatoprotective, neuroprotective, and antiviral characteristics[8]. However, cytotoxic potential of this plant especialy from stem has not been extensively studied and remain unexplored.

So, the objective of the current investigation was extraction, isolation and identification of phytochemicals of *D. erecta* from chloroform extract, and screening of its cytotoxic activity on HL-60 cell lines.

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## MATERIALS AND METHODS

*Materials from Plants*

Plant material was collected from Nanded, Maharashtra, India and Dr. P. Ingale, a botanist from the BSI, Pune, identified the plant.

*Extract Preparation*

The collected stems were dried in an oven at 40˚ for seven days. An electric grinder was used to grind it into a powder. 1 kg powder material was extracted in Soxhlet extractor using chloroform as a solvent. The stem extract was filtered, concentrated, and vacuum-dried at 40ºC using a rotary evaporator and stored in a refrigerator.

*Phytochemical Isolation and Identification using Chromatography and Spectroscopy*

In the present work the bioactive chloroform extract of *D. erecta* was used for the isolation of phytochemicals. Chloroform extract (20 gm) was eluted using a solvent in increasing order of polarity consisting of methanol, chloroform and ethyl acetate over a silica gel in column chromatographic method. Thin-Layer Chromatographic method was used to analyze the fractions and 65 different fractions were collected. Fractions of the extract were collected by using chloroform (F1-27), chloroform: ethyl acetate (F28-40), chloroform: ethyl acetate: methanol (F41-50), and chloroform: methanol (F51-65). Fractions with inseparable chemicals were excluded, while fractions of extract with same TLC patterns were combined. Fractions (F20–24), (F44–47), and (F54–57) were chosen and grouped as Crude Fractions (CF1, CF2, and CF3) based on their significant yield and chromatography. These chosen fractions were subjected to additional purification and chemical separation procedures. Chloroform: ethyl acetate (8:2) and chloroform: ethyl acetate: methanol (8:1.5:0.5) were used in column chromatography to purify crude fractions CF1 and CF2, yielding compounds I and II, respectively. Using silica gel-H and chloroform, ethyl acetate, methanol and acetic acid (0.8:0.5:0.2:0.05) as the mobile phase, Compound III was obtained by preparative TLC of crude fraction CF3.

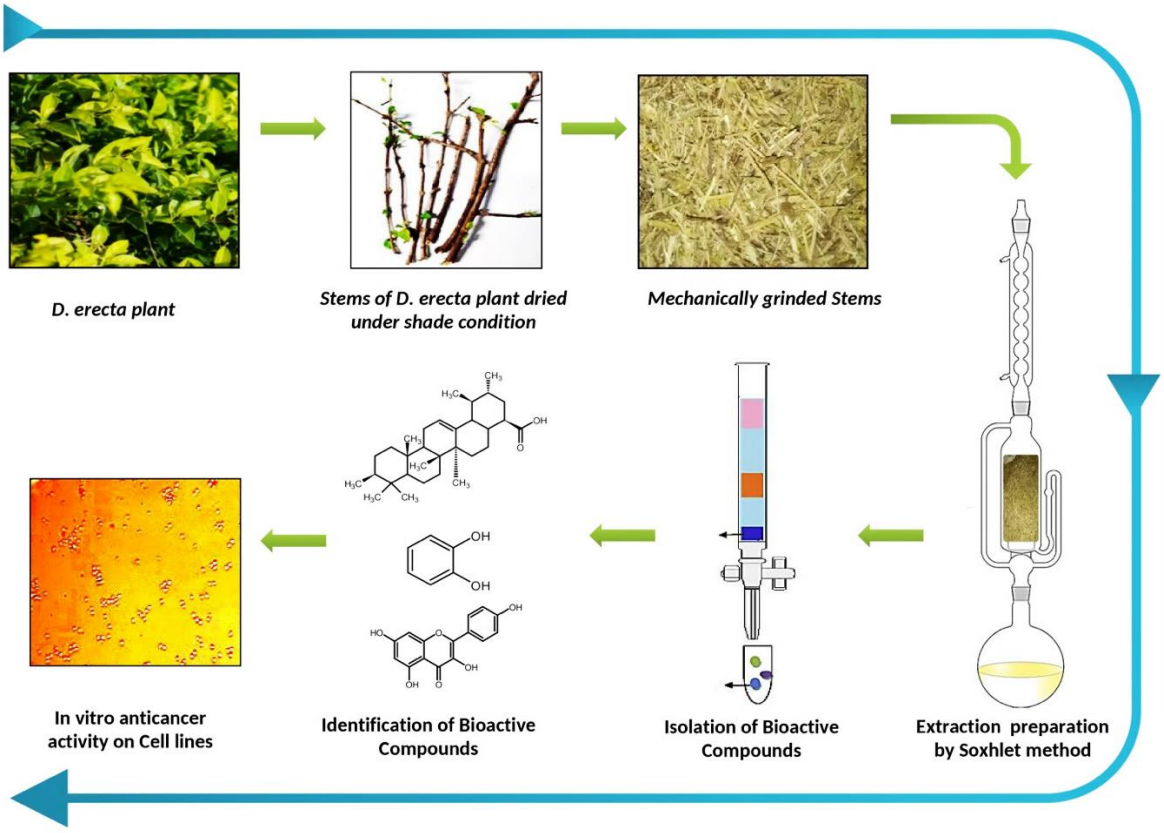
Table 1: Cytotoxic action of compounds isolated from *D. erecta*

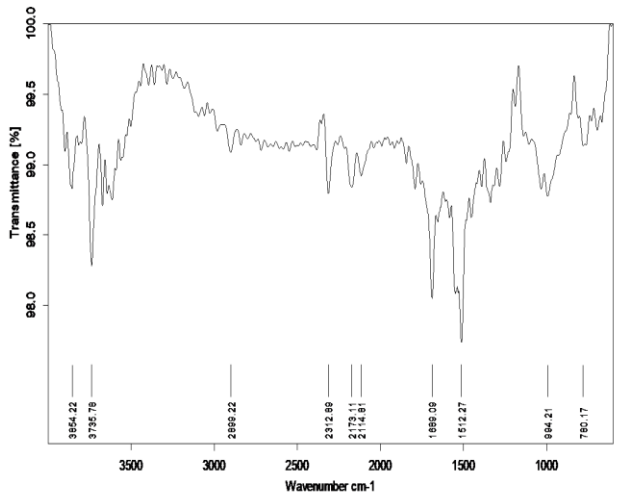
| S.No. | Name of Compound | $IC_{50}$ (µg/ml) |
|---|---|---|
| 1 | Ursolic acid | 98.6 |
| 2 | Pyrocatechol | 164.78 |
| 3 | Kaempferol | 138.57 |
| 4 | Adriyamycine | 30.09 |

The isolated chemicals were identified by using NMR, Fourier transform infrared (FTIR), mass spectroscopy, ultraviolet-visible (UV). The resulting data were then compared with existing reference data.

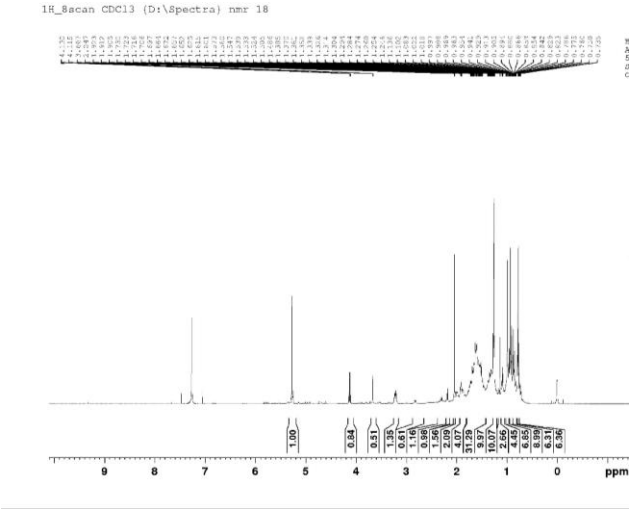
*Assay for Cell Growth Inhibition/Arrest*

Cytotoxic activity of the compounds was screened using SRB assay against cell-lines HL-60. The cells were planted in 96 well plates at a density of 20,000 cells per well in a culture mixture and cultured it in $CO_2$ gas incubator for 24 hrs to perform the SRB experiment. Before being administered to the cell culture media at varying quantities, the chemicals were diluted with the medium and solubilized with Dimethyl Sulphoxide (DMSO). After adding the chemical, incubate the plate in an environment with carbon

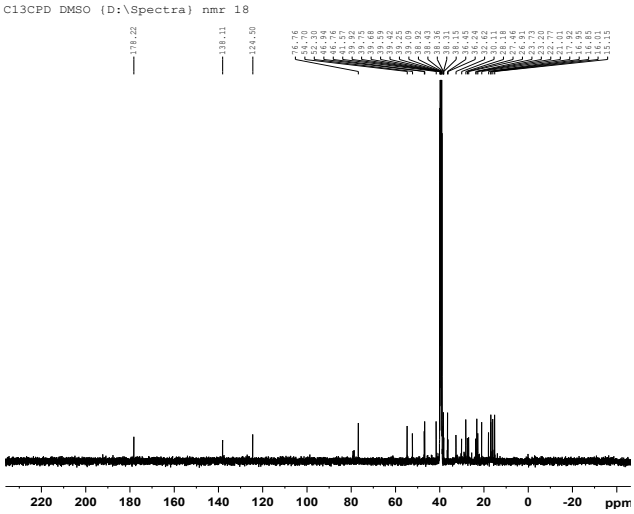




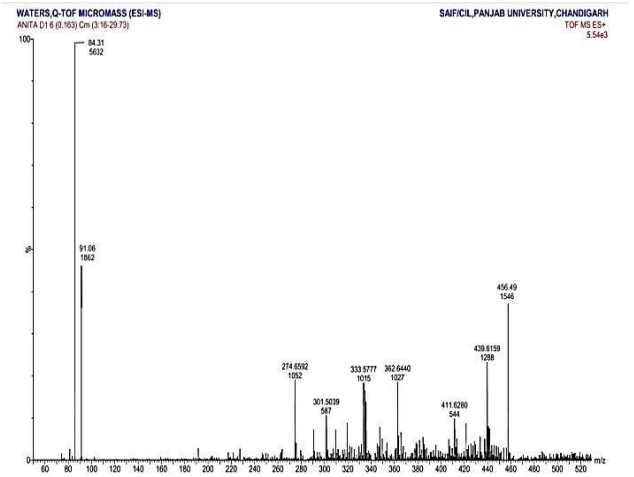

FTIR spectra




$^{1}$H NMR spectra




$^{13}$C NMR spectra




Mass spectra

Figure 1: Spectra of compound I (Ursolic acid)

dioxide (5%) for one day at 37°C. After incubation 50µl Trichloroaceatic acid (30%) was slowly added to the cells to fix them, and incubated at 1 hrs at 4°C.The supernant was rejected and the plate were washed with water and dried. To the each well, SRB solution was added and were incubated at RT. Unbound dye was recovered after staining and by using acetic acid (1%) washing, the residual dye was removed.10 mM trizma base, was used for the elution of bound dye, the absorbance was measured[8-12].

*Statistical Analysis*

Statistical tests, like independent sample one way A N O V A and student t-test, were applied at level of 0.05 for comparisons of anticancer activity of extract and different isolated compounds by using Microsoft Excel data analysis.

**RESULTS AND DISCUSSION**

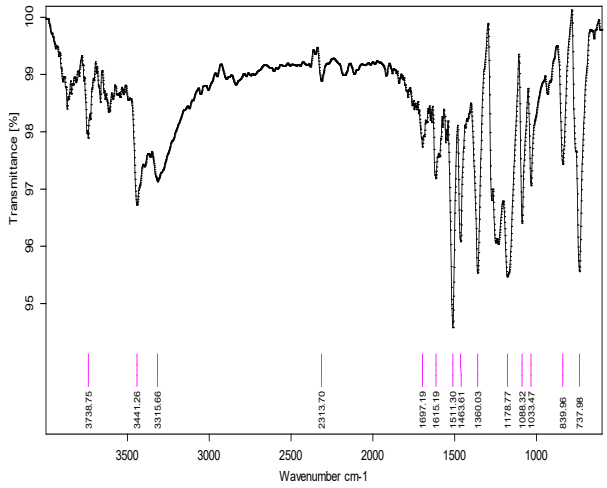
In this study Ursolic acid, Pyrocatechol, and Kaemferol were isolated from the chloroform extract of the *D. erecta* stem (Figure 4). All isolated compounds were identied by using different spectrscopy (UV, M S, FT- IR, $^{1}$H and $^{13}$C N M R) and obtained data were comared with the given literature data. The phtyochemicals identified are,

Compound I (67mg): ($C_{30}H_{48}O_3$) greenish-white, amorphous solid, melting point 270ºC. FTIR spectra exhibited characteristic frequencies at 3635.78 cm $^{-1}$, 2899.22 cm $^{-1}$, 1689.09 cm $^{-1}$,1456.20 cm $^{-1}$,780.17 cm$^{-1}$. IR frequencies at higher wave number i.e. 3635.78 cm$^{-1}$ indicates the presence of -OH group, carboxyl functional group at 1689.09 cm$^{-1}$in the structure, olefinic system (C=C) appeared at 1456.20 cm$^{-1}$& 2899.22 cm$^{-1}$.In Mass spectrum ,the ion ( Molecular) peak appeared at m/z 456[M]$^{+}$ and other peak observed at 439, 411, 362, 333, 301,274, 91, 84 .$^{1}$HNMR spectroscopy showed peaks at δ 0.78 (3H, S , H-23 ) , 0.70 ( 3H, S , H26), 0.86 ( 3H , S , H25), 0.89 (3 H,S, H24) 1.03 (3H, S, H27) , δ 0.82 (H29), 0.91 (H30 ), δ 5.21( 1H,dd,H-12), δ 3.6( 1H,dd, H3), δ 1.55(2H, m, H16), δ 1.58(2H,m, H1), δ 1.54(2H, m, H22), δ 1.4.(2H, m, H2), δ 1.31(2H, m, H21), δ 1.12 (2H, m, H7), δ 1.01(2H, m, H15), δ2.2(2H, dd, H11), δ2.4(1H, d ,H18), δ1.70 (1H,m, H20), δ1.61(1H,m, H-6a), δ1.5(1H,m, H19). $^{1}$H NMR spectrum of compound I exhibited five tertiary methyl groups at 0.86(H-25), δ 0.78 (H-23), 0.70 (H-26), 0.89(H-24) and 1.03(H-27) and two secondary Methyl(-CH3) groups at δ 0.82(H-29) and 0.91 (H-30) and olelifinic

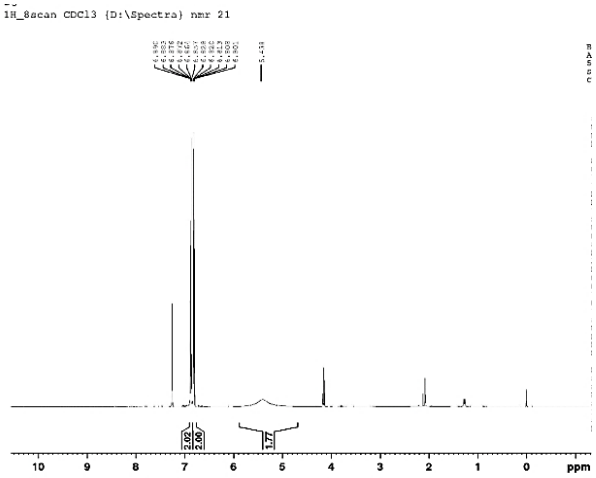
proton at δ 5.21( H-12). The resulting structure was confirmed by $^{13}C$ NMR spectroscopy.$^{13}C$ NMR spectroscopy showed absorption frequencies spectrum at ppm 38.43 (C1), 26.91(C2), 76.76 (C3), 39.09 (C4), 54.70 (C5), 17.92 (C6), 32.62 (C7), 39.75 (C8), 46.76 (C9), 36.45(C10), 23.73 (C11), 124.50 (C12), 138.11 (C13), 41.57 (C14), 27.46 (C15), 23.20 (C16), 52.30 (C17), 46.94 (C18), 39.42 (C19), 39.25(C20), 30.11 (C21), 38.15(C22), 28.18 (C23), 15.15 (C24), 16.01(C25), 16.95 (C26), 22.77 (C27),178.22 (C28), 16.85 (C29), and 21.01 (C30). The $^{13}$CNMR spectra displayed 30 signals, of which seven were methane, nine were methylene, seven were methyl & seven quaternary carbons. The most downfield signals at δ 178.22 were due to carboxylic acid (C-28).The presence of signals at δ 124.50, and δ138.11 shows the presence of unsaturation in the resulting structure. The data obtained are compared with existing data and compound I was identified as Ursolic acid[13-15] (Figure 1).

Compound II (75mg): ($C_6H_6O_2$) off-white to brown crystalline solid, melting point 103ºC. In IR different peaks exhibited at 3441.28 $cm^{-1}$, 3318.15 $cm^{-1}$, 1667.18 $cm^{-1}$, 1611.57 $cm^{-1}$, 1483.00 $cm^{-1,}$ 1170.57$cm^{-1}$, 838.80 $cm^{-1}$. The IR spectrum showed a broad-band at 3441.28 $cm^{-1}$ which indicate the presence of OH Stretching (Hydroxyl) this established the presence of a hydroxyl group in the resulting compound, C=C vibration shown by the peak at 1667.18 cm. MS-spectra shown molecular ion peak at m/z at 110.36, comparable with molecular formula $C_6H_6O_2$. In $^1$H NMR spectra showed absorption peak at δ 5.2 (2H, s, Ar-OH)δ 6.25(2H, m, H-3 & H-6) and δ 6.80 (2H, m, H4 & H5). The $^1$H N M R exhibited typical AA'BB' coupling signals at δ 6.80 (2H, m, H 4, H5), and δ 6.25 (2 H, m, H 3, H6) were assigned to ortho disubstituted benzene ring. The $^{13}$C NMR spectroscopy showed absorption frequencies at δ143.50ppm (C1& C2),121.30 (C3& C6) & δ115.53 (C4& C5). The absorption frequencies demonstrated by the spectrum at δ 143.50, 121.30, 115.53 indicated symmetry in structure. The spectral established data and further comparison of the collective data given in the literature survey, compound II was confirmed i.e Pyrocatechol[16-18] shown in Figure 2.

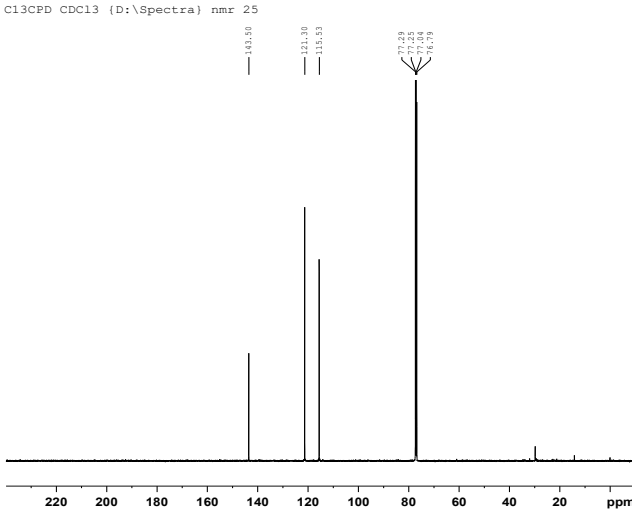
Compound III (40mg): ($C_{15}H_{10}O_6$) Yellowish amorphous solid substance, melting point 287ºC. The IR spectra exhibited band at 3710.33$cm^{-1}$, 1655.80$cm^{-1}$, 1500.49$cm^{-1}$,



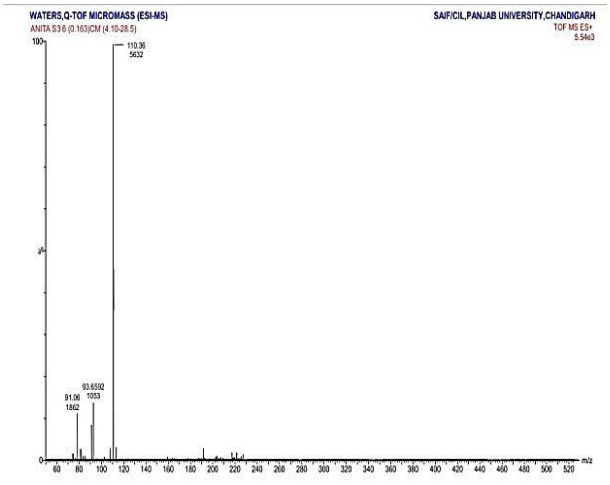

FTIR spectra




$^1$H NMR spectra

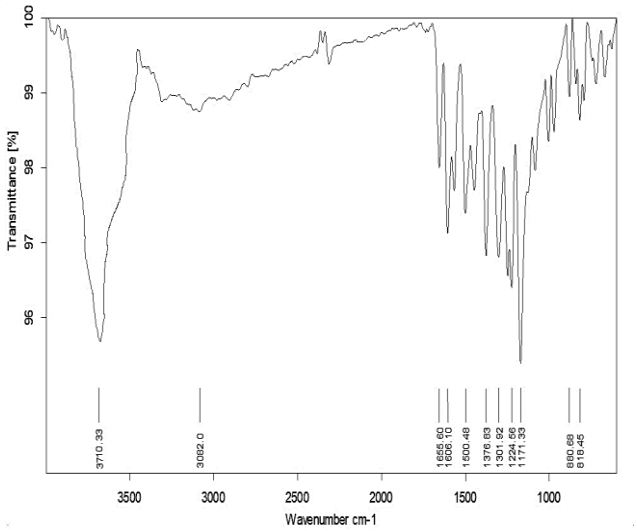



$^{13}$C NMR spectra

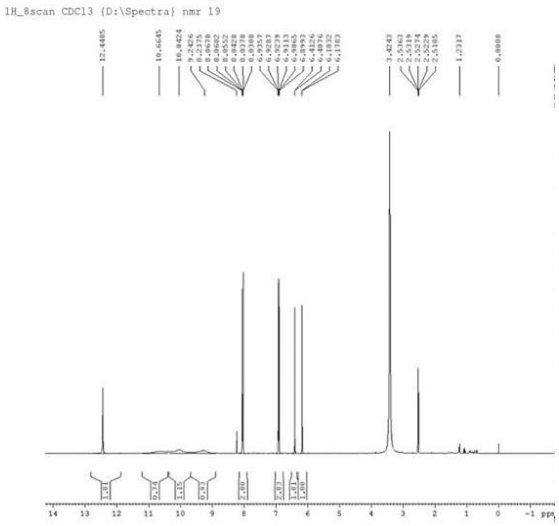



Mass spectra

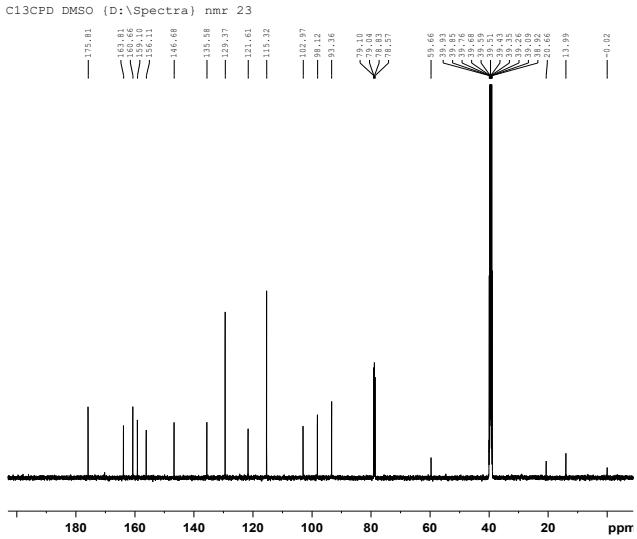
Figure 2: Spectra of compound II (Pyrocatechol)



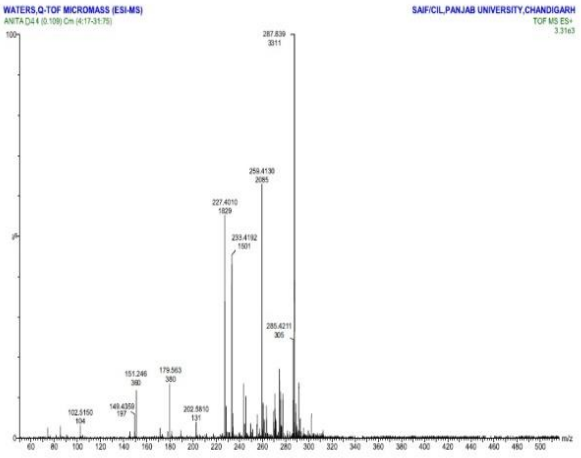

FTIR spectra




$^{1}$H NMR spectra

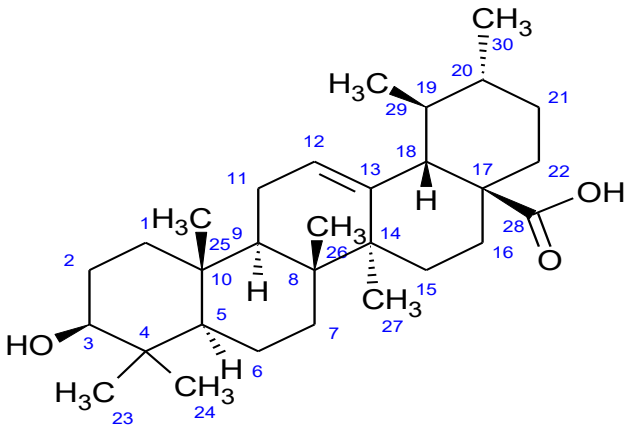



$^{13}$C NMR spectra

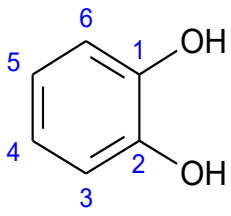



Mass spectra

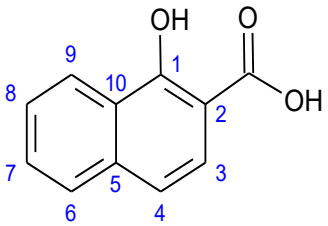
Figure 3: Spectra of compound III (Kaempferol)




Compound I: Ursolic Acid




Compound II: Pyrocatechol




Compound III: Kaempferol

Figure 4: Structures of the isolated compounds

1301.92cm$^{-1}$, 1171.53 cm$^{-1}$, 1224.56 cm$^{-1}$ and 890.68 cm$^{-1}$. Due to the hydroxyl group,at 3710.33 cm$^{-1}$ showed the band in IR spectra, a peak at 1301.92 cm$^{-1}$ due to (C –O- C) groups and1655.60 cm$^{-1}$ due to C=O stretch.

Mass spectrum shows molecular ion peaks at m/z 286 $[M]^{+}$ & molecular formula ($C_{15}H_{10}O_6$), other peaks observed at 252, 233, 227, 202,179.151,107 .$^{1}$H NMR spectrum showed a peak at δ 6. 01 (1 H , d, H 6), δ 6.56 (1H, d, H-8), δ8.30

(2H, d, H-2' and H-6'), δ6.80(2H, d, H-3' & H-5') . spectrum showed the presence of two protons at δ6.01 (1H, d, H-6) and 6.56 (1H, d, H-8), NMR spectra exhibited the existance of two doublet signals at δ 8.30 (2H , d, H 2' and H 6'), and 6.80 (2H , d , H 3' & H5 ') due to four protons in ring (aromatic B) characteristic for 1',4'- disubstituted flavones.[13]C NMR spectra showed absorption frequencies at ppm 175.81(C4), 162.81.76 (C7). 160.66 (C5), 159.10 (C4'), 156.11 (C9), 146.68 (C2), 135.58(C3), 129.37 (C2' & C6'), 121.61 (C1'), 115.32 (C3' & C5'), 102.97(C10), 98.12(C6), 93.86 (C-8). [13]C NMR spectra absorption spectra shown a total of fifteen carbon signals. Signals at C-4 at δ175.81 shows presence of a carbonyl CO functional group in resulting structure. Data obtained from spectrum & comparison with the reported data, (Figure 3) the compound III, was established as Kaempferol[19-21] (Figure 3).

Deprived selection of compounds and all side effects of the present anti-cancer drugs have need for the advancement of novelty, safer as well as effective anticancer substance or agents. traditional medical , biodiversity and knowledge had gives necessary lead compounds for cancer therapy, proved by the discovery of the alkaloids from vinca[22].

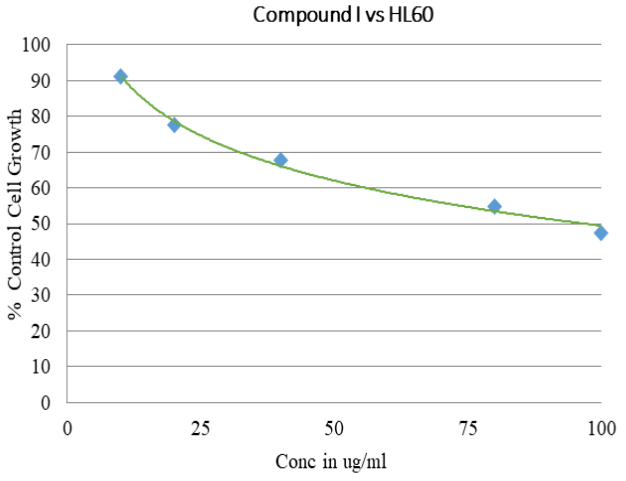



Figure 5: Cytotoxic Effect of compound I (Ursolic acid) on HL-60 cell lines growth

Different *in-vitro* methods are useful for the preliminary investigation of anticancer compounds from plants[22-25]. The use of cell lines has been routinely carried out to screen the active compounds from natural sources. The isolated compounds Ursolic acid (I) Pyrocatechol (II) and Kaemferol (III) were screened for cytotoxicity using SRB assay against HL60 cell lines. Percent growth of cell control and % growth of inhibition were calculated.

The $IC_{50}$ value of isolated compounds was determined by using a linear line regression equation formula and found to be 98.6µg/ml (Ursolic acid), 138.57µg/ml (Pyrocatechol) and 164.74µg/ml (Kaempferol). All compounds showed dose-dependent cytotoxic activity on the HL-60 cell line (Fig.5) but its lower which was compared to positive reference control Adriamycin (10.09µg/ml). Derivatives of Ursolic acid are reported to possess *in vivo* and *in vitro* anticancer activity[26,27]. Among the isolated compounds, Ursolic acid was the most active cytotoxic constituent withan $IC_{50}$ value be 98.6µg/ml. . (Table 1 and Figure 5,6 &7) The cytotoxic activity of extract was statistically different ( $P < 0.05$) as comparing to the positive(+ve) control. Moreover, cytotoxic action of Ursolic acid was statistically different ($P<0.05$) than Pyrocatechol and Kaempferol when evaluated by one way ANOVA.

## CONCLUSION

The present research extracted, isolated and identified phytochemicals from the stem of *Duranta erecta* and screened for *in-vitro* cytotoxic action against $HL_{60}$ cell lines. Different analytical tools were employed for the identification of three phytochemical such as compounds Ursolic acid, Pyrocatechol, and Kaempferol. All isolated compounds showed dose dependent cytotoxic action against HL-60 cell lines. Amongst them, ursolic acid exhibited greater cytotoxic activity than other two isolated compounds. Besides, the cytotoxic activity of the extract was significantly greater than positive control. This research provides the significance of herbal bioactives for the development of effective cancer therapies and offer roadmap for future research aimed at finding newer therapeutic agents for cancer treatment.

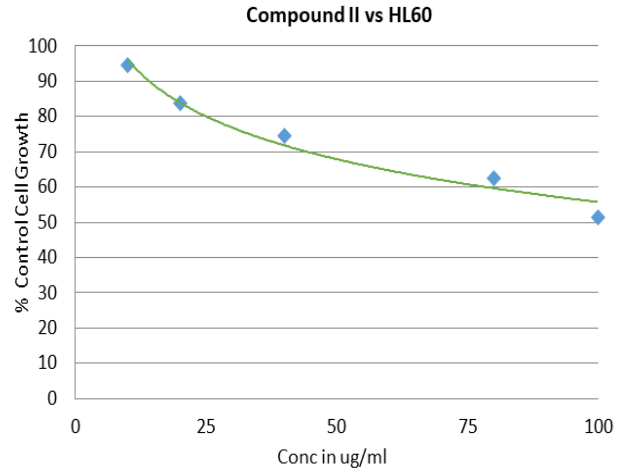



Figure 6: Cytotoxic Effect of compound II (Pyrocatechol ) on HL-60 cell lines growth

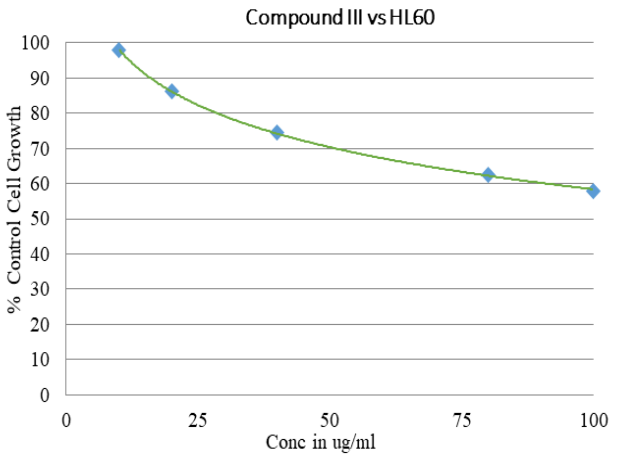



Figure 7: Cytotoxic Effect of compound III (Kaempferol) on HL-60 cell lines growth

**REFERENCES**


1. Chaurasia V, Arora N, Arora P. Pharmacokinetic Studies and Evaluation of Nanoparticulate Drug Delivery System in the Treatment of Cancer. International Journal of Drug Delivery Technology. 2024;14(4):2331-35. doi: 10.25258/ijddt.14.4.54.
2. Singh C , Ahuja D, Mehta S C, *in vivo* anticancer activity of Cleome viscose Linn. alcoholic extract and its fractions against Ehrlich's ascites carcinoma (EAC) cell Line. International Journal of Pharmaceutical Quality Assurance 2019; 10(2); 256-63 doi: 10.25258/ijpqa.10.2.7.3.
3. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2024;74(3):229-63.
4. Arruebo M, Vilaboa N, Sáez-Gutierrez B, Lambea J, Tres A, Valladares M, González-Fernández Á. Assessment of the evolution of cancer treatment therapies. Cancers. 2011 ;3(3):3279-330. doi: 10.3390/cancers3033279.
5. Zahreddine H, Borden K L. Mechanisms and insights into drug resistance in cancer. Frontiers in Pharmacology. 2013;4:28. doi: 10.3389/fphar.2013.00028.
6. Imtiaz I, Schloss J, Bugarcic A. Traditional and contemporary herbal medicines in management of cancer: A scoping review. Journal of ayurveda and integrative medicine. 2024;15(1):100904. doi: 10.1016/j.jaim.2024.
7. Dombe S A, Shirote P J. Isolation, In-silico Studies, and Biological Evaluation of Higenamine from Annona squamosa L. against Breast Cancer. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):1039-1047.
8. Srivastava M, Shanker K. *Duranta erecta* Linn: A critical review on phytochemistry, traditional uses, pharmacology, and toxicity from phytopharmaceutical perspective. Journal of Ethnopharmacology. 2022;293:115274. doi: 10.1016/j.jep.2022.
9. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature protocols. 2006;1(3):1112-6. doi: 10.1038/nprot.2006.
10. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI: Journal of the National Cancer Institute. 1990;82(13):1107-12. doi: 10.1093/jnci/82.13.1107.
11. Samiya, Saha S, Jakhmola V, Gairola N, Singh M. Molecular Docking, Synthesis, Antiproliferative Activity against MCF-7, and *In-vitro* Alpha Amylase Activities of Newer Generation Pyrimidino Hydroxamic Acid Derivatives. International Journal of Drug Delivery Technology. 2024;14(2):649-659.
12. El Semary NA, Fouda M. Anticancer activity of Cyanothece sp. strain extracts from Egypt: First record. Asian Pacific Journal of Tropical Biomedicine. 2015;5(12):992-5.
13. Mrutyunjaya Rao R, Ramakrishna K, Suresh Babu K, Surya Kumar MV. Isolation of Urosilic Acid from *Knoxia corymbosa.* Natural Products Chemistry & Research. 2017;5:1-3. 10.4172/2329-6836.1000286
14. Ladda PL, Magdum CS. Antitubercular activity and isolation of chemical constituents from plant Vitex negundo Linn. Iranian Journal of Pharmaceutical Research. 2018;17(4):1353.
15. Batra A, Sastry VG. Extraction of ursolic acid from *Ocimum sanctum* and synthesis of its novel derivatives: Effects on extracellular homocysteine, dihydrofolate reductase activity and proliferation of HepG2 human hepatoma cells. Pteridines. 2013;24(3-4):191-9. DOI 10.1515/pterid-2013-0023.
16. Nair PR, Melnick SJ, Wnuk SF, Rapp M, Escalon E, Ramachandran C. Isolation and characterization of an anticancer catechol compound from *Semecarpus anacardium.* Journal of ethnopharmacology. 2009;122(3):450-6.
17. Sundaram R, Muthu K, Nagaraj S, Shanthi P, Sachdanandam P. Isolation and characterization of catechol derivatives from Semecarpus anacardium seeds and their antibacterial potential in *in vitro.* Biomedicine & Preventive Nutrition. 2014;4(2):177-80.10.1016/j.bionut.2013.12.001.
18. Huang S, Zhang CP, Li GQ, Sun YY, Wang K, Hu FL. Identification of catechol as a new marker for detecting propolis adulteration. Molecules. 2014;(7):10208-17. doi.org/10.3390/molecules190710208.
19. Aisyah LS, Yun YF, Herlina T, Julaeha E, Zainuddin A, Nurfarida I, Hidayat AT, Supratman U, Shiono Y. Flavonoid compounds from the leaves of *Kalanchoe prolifera* and their cytotoxic activity against P-388 murine leukimia cells. Natural Product Sciences. 2017;23(2):139-45. doi.org/10.20307/nps.2017.23.2.139.
20. Feng W, Hao Z, Li M. Isolation and structure identification of flavonoids. Flavonoids-From Biosynthesis to Human health/Ed. by Justino GC. Intech. 2017;17-43. doi:10.5772/67810.
21. Nur HA, Fatima S, Rohaya A. Isolation of flavonols from the stems of Malaysian Uncaria cordata var. ferruginea (Blume) Ridsd. Malaysian Journal of Analytical Science. 2016;20(4):844-8.
22. Noble RL. The discovery of the vinca alkaloids—chemotherapeutic agents against cancer. Biochemistry and cell biology. 1990 Dec 1;68(12):1344-51. PMID: 2085431.
23. Raut DN, Patil TB, Chaudhari SR, Pal SC, Mandal SC. Antimitotic effect of ethanol fraction of Hibiscus mutabilis leaf and flowers. Research in Pharmacy. 2014;4(5):16.
24. Aslantürk OS. *In vitro* cytotoxicity and cell viability assays: principles, advantages, and disadvantages. Genotoxicity-A Predictable Risk to Our Actual World. 2018;2:64-80.
25. Ogbole OO, Segun PA, Adeniji AJ. *In vitro* cytotoxic activity of medicinal plants from Nigeria ethnomedicine

on rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. BMC Complementary and Alternative Medicine. 2017;17:1-0. DOI: 10.1186/s12906-017-2005-8

26. Shao JW, Dai YC, Xue JP, Wang JC, Lin FP, Guo YH. *In vitro* and *in vivo* anticancer activity evaluation of ursolic acid derivatives. European Journal of Medicinal Chemistry. 2011;46(7):2652-61.doi: 10.1016/j.ejmech.2011.03.050
27. Kalani K, Yadav DK, Khan F, Srivastava SK, Suri N. Pharmacophore, QSAR, and ADME based semisynthesis and *in vitro* evaluation of ursolic acid analogs for anticancer activity. Journal of Molecular Modeling. 2012;18(7):3389-413.