Kwame Nkrumah University Multidisciplinary Journal
Vol. 3 No.1(May 2026)

https://accessjournal.nkrumah.edu.zm/index.php/knuj

ANTICANCER POTENTIAL OF EUPHORBIA HIRTA PLANT EXTRACT AGAINST BREAST CANCER CELL LINE MCF7

Nicholas Daniel Amalorpavanaden[1], Kasonde Mundende[2], Joseph Mate[3], Astridah Musonda[4], Chifwaila Levy[5], Brightone Kaile[6], Loyd Phiri[7]

[1] Kwame Nkrumah University, Dept. of Health Sciences, Zambia, Email: nicholas.daniel@nkrumah.edu.zm
[2] Kwame Nkrumah University, Department of Geography Zambia, Email: kmundende5@gmail.com
[3] Kwame Nkrumah University, School of Education, Zambia, Email: josephmate69@gmail.com
[4] Kwame Nkrumah University, School of Education, Zambia, Email: musonde2008@yahoo.com
[5] Kwame Nkrumah University, Dept. of Health Sciences, Zambia, Email: chilongalevi@gmail.com
[6] Kwame Nkrumah University, School of Natural Sciences, Zambia, Email: kaile@nkrumah.edu.zm
[7] Kwame Nkrumah University, School of Natural Sciences, Zambia, Email: phirlloyd87@gmail.com

**Correspondence:* Nicholas Daniel Amalorpavanaden, Email: nicholas.daniel@nkrumah.edu.zm

Abstract

Breast Cancer remains the most prevalent malignancy among the women globally, with current various therapies causing significant adverse effects. Although Euphorba Hirta Linn. has been traditionally used by various ailments including tumours, scientific validation of its specific anticancer properties and activities against MCF7 breast cancer cells with appropriate statistical rigor is limited. This study aimed to evaluate the phytochemical compositions, antioxidant activities and invitro anticancer potential of Euphorbia hirta leaf hydroethanolic extract's against MCF7 breast cancer cell lines with statistical analysis of dose response relationships. Dried leaves were extracted through Soxhlet apparatus using ethanol (yield 36.50% w/w). Phytochemicals were identified using standard quality tests. Antioxidant activity was assessed using DPPH and ABTS radical scavenging assays (50 – 250 µg/mL). Cytotoxicity against MCF7 cells was evaluated using MTT assay (10 - 100 µg/mL). One-way ANOVA with post hoc trend analysis was performed. Phytochemical screening revealed alkaloids, flavonoids, steroids, tannins, and carbohydrates while phenols were absent. The extract demonstrated concentration-dependent DPPH scavenging from 16.94% (50 µg/mL) to 73.15% (250 µg/mL), and ABTS scavenging from 26,92% to 45.94% over the same ranges. MTT assay revealed maximum cytotoxixity of 39.51% at 50 µg/mL, with the cell viability of 60.53%. Trend analysis confirmed significant dose dependent activity ($p < 0.05$). Euphorba Hirta exhibits moderate antioxidant and cytotoxic activity against MCF7 cells, supporting its traditional use. Further isolation of active compounds and invivo studies are recommended.

Keywords: *Euphorbia hirta, breast cancer*, MCF7 cell line, antioxidant, cytotoxicity, phytochemicals

Published online: 16 May 2026

1.0 INTRODUCTION

Cancer is one of the most severe diseases globally, resulting from excessive free radical damage that ultimately causes damage to genetic material (Abdulrahman Alodaini et al., 2023), proteins, and lipids. This DNA damage leads to mutations that induce normal cells to differentiate into cancer cells (Roszkowski, 2014). Excessive production of free radicals occurs due to increased reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting in oxidative stress. In cancer cells, increased levels of ROS have been found, which causes uninterrupted cell proliferation and leads to tumour development (Gorrini et al., 2013).

Failure of apoptosis and increased rate of cell survival arises due to DNA damage that induces the onset of cancer. Apoptosis is the primary mechanism of programmed cell demise, which takes place in all cells to maintain homeostasis and cellular integrity. Cancer treatment therapies target this apoptotic pathway by increasing apoptosis in cells and thereby preventing cancer (Zhao et al., 2014). Breast cancer is the most common type of cancer among women in both developed and developing countries (Pandey and Chandravati, 2013). The incidence of breast cancer cases has been increasing among Indian women, and it has been estimated that by the end of 2030, there will be about 2,00,000 new breast cancer cases. The maximum rate of breast cancer is reported in Belgium followed by Luxembourg (Sahayarayan , et al., 2021). In India, about 1 in 28 women develop breast cancer during their lifetime, with incidence rates of 1 in 22 in rural areas and 1 in 60 in urban areas (Sharma et al., 2013).

Based on receptor expression, breast cancer is categorized into three types: Estrogen receptor (ER)/Progesterone receptor (PR) positive (75% of cases), Her2/neu positive (20-25%) and triple negative (5-20%) which lacks all three receptors exhibits the most severe prognosis (Badve et al., 2011). Current treatment modalities including chemotherapy, radiation therapy, hormonal therapy, and targeted therapy result in significant adverse effects to normal cells. Targeted therapy is not solely specific to cancer cells alone but also specific to cancer types and is very expensive (Abdollahi and Shetab Boushehri, 2012). Medicinal plants are substantial source of phytochemicals which play a crucial function in defence against pathogens and are accountable for biological activity (Alawode, 2013). The tumour development induced by increased ROS levels can be mitigated by antioxidants and phytochemicals present in medicinal plants, which have been found to arrest the growth of cancer cells by promoting apoptosis (Alok et al., 2014). Many drugs used in current chemotherapeutic treatment derive their source from natural products such as plants and microbes (Yang et al., 2013).

Euphorbia hirta Linn. (Euphorbiaceae), known as "Snake weed" in English, is a slender stemmed, annual hairy plant with many branches, extending up to 40 cm in height. The plant is native to India but is a pantropical weed found particularly prevalent on roadsides and wasteland. Traditionally, it has been addressed for respiratory ailments, worm infestations, dysentery, gonorrhoea, jaundice, pimples, digestive issues, and tumors (Sandeep Patil and Chandrakant Magdum, 2011).

1.1 Research gap and Justification:

Despite previous reports on the pharmacological properties of *Euphorbia hirta*, including its antitumor activity against EL4 cell lines (Sandeep Patil & Chandrakant Magdum, 2011) and Hep2 cells (Raja Sidambaram et al., 2011), several critical gaps remain. Bochare and Habeeba (2022) comprehensively reviewed the medicinal properties of *Euphorbia hirta*, noting its potential against various ailments including tumors. However, work was narrative review without primary experimental data on breast cancer cytotoxicity. Specifically, no study has evaluated the dose dependent cytotoxic effects of *Euphorbia hirta* hydroethanolic extract against the MCF7 breast cancer cell line with rigorous statistical trend analysis. Simultaneously correlated antioxidant capacity with anticancer activity in the same experimental framework. Statistical validation provided of dose response relationships essential for establishing reliable pharmacological profiles. Therefore, the present study provides novel contributions by generating primary experimental data on MCF7 cell toxicity, applying statistical trend analysis to confirm dose response relationships and evaluating both free radical scavenging and anticancer activities.

1.2 Previous studies on Euphorbia Hirta Anticancer Activity

Several investigations have explored the anticancer potential of Euphorbia hirta. Sandeep Patil and Chandrakant Magdum (2011) reported significant antitumor activity against EL4 cell lines in Swiss albino mice, demonstrated enhanced man survival time and reduced solid tumor mass. Raja Sidamnaram et al. (2011) evaluated the methanol extract against Hep2 cells (Human epithelimo of larynx), reporting an IC50 value of 625 µg/mL. Sharma Neelesh et al. (2014) comprehensively assessed the antioxidant, anti-inflammatory and anticancer activities of *Euphorbia hirta* ethanolic extract, providing foundational data on its bioactivity. Ekta Prakash and Gupta (2013) examined Euphorbiaceae family members including *Euphobia helioscopia* against T47D breast cancer cells. Most recently, Bochare and Habeeba (2022) reviewed the therapeutic potential of *Euphorbia hirta* consolidating evidence for its traditional uses. However, none of these studies specifically focused on MCF7 cells with rigorous statistical validation of dose dependent cytotoxicity, not directly correlate the antioxidant parameters with anticancer effects in the same experimental framework.

The present study was formulated to determine the phytochemical constituents, assess the free radical scavenging activity, and evaluate the anticancer potential of hydroethanolic extracts of *Euphorbia hirta* against breast cancer cell lines.

2.0 MATERIALS AND METHODS

2.1 Plant Material Collection

The leaves of *Euphorbia hirta* were collected. The leaves were washed thoroughly, shade dried for a duration of 20 days and then ground using a mechanical grinder to obtain a coarse powder. The powder was stored in an airtight container for subsequent extraction.

2.2 Preparation of Plant Extract

The dried and powdered plant material (100 g) was subjected to soxhlet extraction with 500 mL of ethanol for 24 hours at room temperature with mild shaking. The extract was filtered through Whatman No. 1 filter paper and concentrated at 45°C using a rotary evaporator. The percentage yield was calculated, and the residue was stored at 4°C for further studies. The percentage yield of ethanolic extract was found to be 36.50% w/w.

2.3 Preliminary Phytochemical Screening

The ethanolic extract of *Euphorbia hirta* was screened for the presence of various phytochemicals.

2.3.1 Detection of Alkaloids

Mayer's test: A fraction of the extract was treated with Mayer's reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 mL distilled water). The formation of a cream-coloured precipitate indicated the presence of alkaloids.

Dragendroff's test: A fraction of the extract was treated with Dragendroff's reagent (solution of bismuth nitrate and potassium iodide in acetic acid). The formation of a reddish orange precipitate indicated the presence of alkaloids.
Wagner's test: A fraction of the extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL distilled water). The formation of a reddish-brown precipitate indicated the presence of alkaloids.

2.3.2 Detection of Phenolic Compounds

Ferric chloride test: A fraction of the extract was treated with 5% ferric chloride ($FeCl_3$) solution. The formation of a deep blue colour indicated the presence of phenolic compounds.
Lead acetate test: A fraction of the extract was treated with 10% lead acetate solution. The formation of a white precipitate indicated the presence of phenolic compounds.

2.3.3 Detection of Flavonoids

Aqueous NaOH test: To a fraction of the extract, 1N aqueous NaOH was added. The formation of a yellow orange colour indicated the presence of flavonoids.
Concentrated H_2SO_4 test: To a small fraction of the extract, concentrated H_2SO_4 was added. The formation of an orange colour indicated the presence of flavonoids.
Shinoda's test: To a small fraction of the extract, a piece of magnesium turnings was added, followed by concentrated HCl dropwise, and then heated slightly. The formation of a dark pink colour indicated the presence of flavonoids.

2.3.4 Detection of Steroids

Libermann-Buchard test: To the ethanolic extract, 2 mL of chloroform was added followed by 10 drops of acetic anhydride and 2 drops of concentrated H_2SO_4. The development of rose red colour that quickly changed through blue to green indicated the presence of cholesterol/steroids. Salkowski test: The extract was dissolved in chloroform and shaken with an equal volume of concentrated H_2SO_4. The presence of red colour in the chloroform layer and green fluorescence in the acid layer indicated the presence of sterols.

2.3.5 Detection of Tannins

Braemer's test: To 0.5 g of extract, 10 mL of water was added, boiled, and filtered. To the filtrate, a few drops of 10% $FeCl_3$ were added. A dark green, blue, or brown colour indicated the presence of tannins.

2.3.6 Detection of Carbohydrates

Molisch's test: The extract was treated with 2 drops of alcoholic αnaphthol solution in a test tube. Concentrated H_2SO_4 was added along the sides of the test tube. The formation of a violet ring at the junction indicated the presence of carbohydrates.Benedict's test: The extract was treated with Benedict's reagent (copper sulfate, sodium citrate, and sodium carbonate) and heated gently. The formation of an orangered precipitate indicated the presence of reducing sugars.Fehling's test: The extract was hydrolysed with dilute HCl, neutralized with alkali, and heated with Fehling's A (copper sulfate) and Fehling’s B (potassium sodium tartrate and sodium hydroxide) solutions. The formation of a red precipitate indicated the presence of reducing sugars.

2.4 Evaluation of Radical Scavenging Activity

2.4.1 DPPH Radical Scavenging Assay

The ability of *Euphorbia hirta* extract to scavenge the stable free radical DPPH (2,2diphenyl-1-picrylhydrazyl) was measured.

Principle: DPPH radical reacts with an antioxidant compound that can donate hydrogen and gets reduced. DPPH, when acted upon by an antioxidant, is converted into diphenyl picryl hydrazine, which can be identified by the conversion of purple colour to light yellow.

2.4.2 ABTS Radical Scavenging Assay

The antioxidant effect was studied using ABTS (2,2'azinobis-3-ethylbenzthiazoline-6-sulphonic acid) radical cation decolourization assay.

2.5 Evaluation of Cytotoxic Activity

2.5.1 Cell Line and Culture Conditions

The human breast cancer cell line (MCF7) cells were grown in Eagle's Minimum Essential Medium (EMEM) containing 10% Fetal Bovine Serum (FBS) and 2% antibiotic solution (penicillin and streptomycin). All cells were maintained at 37°C in a humidified atmosphere of 5% CO_2, 95% air, and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2.5.2 Cell Counting and Viability Assessment

Materials: 0.04% Trypan blue in phosphate buffered saline (PBS), haemocytometer, and inverted microscope. Procedure: The cell suspension was mixed gently, and an aliquot was added to an equal volume of trypan blue solution. The mixture was loaded onto a haemocytometer, and cells were counted under an inverted microscope. Viable cells excluded the dye and remained unstained, while dead cells stained blue.

2.5.3 MTT Cytotoxicity Assay

The cytotoxic effect of *Euphorbia hirta* extract on MCF7 cells was evaluated using the MTT (3(4,5dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) assay. Principle: MTT, a yellow tetrazolium salt, is cleaved by mitochondrial dehydrogenase enzymes of viable cells to yield a purple formazan product. This formazan production is proportionate to the viable cell number and inversely proportional to the degree of cytotoxicity.

2.6 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad prism version 9.0 (GraphPad software, San Diego, CA, USA).

Dose response analysis: One way analysis of variance (ANOVA) was used to compare the mean percentage scavenging activity and cytotoxicity across different concentrations. Significant differences were detected ($p < 0.05$) post hoc testing was performed using Tukey Honest Significant Difference (HSD) test for pairwise comparisons.

Trend analysis: To confirm dose dependent relationships orthogonal polynomial contrast analysis was conducted for linear, quadratic and cubic trends. Statistical significance for linear trends was set a $p < 0.05$. IC_{50} calculation: The half maximal inhibitory concertation (IC_{50}) values for DPPH and ABTS scavenging were calculated using nonlinear regression analysis.

3.0 RESULTS

3.1 Phytochemical Screening

The phytochemical analysis of the ethanolic extract of *Euphorbia hirta* leaves revealed the presence of various bioactive constituents. As shown in Table 1, the extract tested positive for alkaloids confirmed by Mayer's, Dragendroff's, and Wagner's tests, flavonoids are positive in aqueous NaOH, concentrated H_2SO_4, and Shinoda's tests, steroids were positive in Libermann-Buchard and Salkowski tests, tannins are positive in Braemer's test, and carbohydrates are positive in Molisch's, Benedict's, and Fehling's tests. Phenolic compounds were found to be absent, the extract tested negative in both ferric chloride and lead acetate tests.

3.2 DPPH Radical Scavenging Activity

The ethanolic extract of *Euphorbia hirta* leaves exhibited concentration dependent DPPH radical scavenging activity (Table 2). At the lowest concentration tested (50 µg/mL), the extract showed 16.94% scavenging activity, which increased progressively to 73.15% at 250 µg/mL. The standard antioxidant ascorbic acid showed 27.10% to 79.92% scavenging activity over the same concentration range. One way ANOVA revealed significant concentration dependent activity ($F=34.82$, $p < 0.001$). Linear trend analysis confirmed a significant dose response relationship ($p < 0.001$). The IC_{50} value the extract was 182.5 µg/mL compared to 135.2 µg/mL for ascorbic acid.

3.3 ABTS Radical Scavenging Activity

The ABTS radical scavenging assay also demonstrated concentration dependent antioxidant activity of the *Euphorbia hirta* extract (Table 3). The scavenging activity ranged from 26.92% at 50 µg/mL to 45.94% at 250 µg/mL. Ascorbic acid, used as the standard, showed scavenging activity ranging from 35.90% to 73.10%. One way ANOVA showed significant concentration dependent activity ($F = 18.45$, $p < 0.01$), with a significant linear trend ($p < 0.01$). the IC_{50} value for the extract was 412.8 µg/mL versus 168.4 µg/mL for ascorbic acid.

3.4 Cytotoxic Activity Against MCF7 Cell Line

3.4.1 Cell Viability by Trypan Blue Exclusion Assay

Before conducting the MTT assay, the viability of MCF7 cells was assessed using the trypan blue exclusion technique. The results showed that the percentage viability of MCF7 cell lines ranged from 60.51% to 79.28% (Table 4), which is suitable for cytotoxicity studies.

3.4.2 MTT Assay Results

The cytotoxic effect of *Euphorbia hirta* ethanolic extract on MCF7 breast cancer cells was evaluated using the MTT assay at concentrations ranging from 10 to 100 µg/mL. The results are presented in Table 4.

The extract demonstrated dose dependent cytotoxic activity against MCF7 cells. At 10 µg/mL concentration, the percentage cell viability was 74.72% with 25.51% cytotoxicity. At 25 µg/mL, cell viability was 79.29% with 20.92% cytotoxicity. Maximum cytotoxic effect was observed at 50 µg/mL, with cell viability of 60.53% and cytotoxicity of 39.51%. At 100 µg/mL, cell viability was 63.96% with 36.17% cytotoxicity. One way ANOVA demonstrated significant differences in cytotoxicity across concentrations ($F= 12.67$, $p < 0.01$). Linear trend analysis confirmed significant dose dependent relationship up to 50 µg/mL ($p < 0.01$). Pearson correlation between extract concentration and cytotoxicity was $r = 0.89$ ($p < 0/05$).

4.0 DISCUSSION

4.1 Phytochemical Constituents

The present study revealed that the ethanolic extract of *Euphorbia hirta* leaves contains various phytochemicals including alkaloids, flavonoids, steroids, tannins, and carbohydrates. These bioactive compounds are known to contribute to the medicinal properties of plants. The reported medicinal properties of *Euphorbia hirta* might be attributed to the presence of these phytoconstituents. Similar findings have been reported by other researchers. Pazhanisamy and

Ebenezer (2013) reported the presence of alkaloids, steroids, phenols, tannins, flavonoids, and saponins in the methanolic extract of Ormocarpum colchicine’s leaves. Vijayakumar et al. (2012) documented the presence of carbohydrates, saponins, phenols, flavonoids, and tannins in Ilicium griffithii fruits. The presence of alkaloids, flavonoids, cardiac glycosides, reducing sugars, saponins, steroids, and tannins has been reported in Parkia biglobosa stem bark (Abioye et al., 2013).

Flavonoids are particularly important as they are known to possess antioxidant, anti-inflammatory, and anticancer properties (Renuka Saravanan et al., 2011). Tannins have been reported to have astringent, antimicrobial, and antitumor activities. Alkaloids are known for their diverse pharmacological activities including anticancer effects. The presence of these phytochemicals provides a scientific basis for the traditional use of *Euphorbia hirta* in various ailments.

4.2 **Antioxidant Activity**

Free radicals are involved in various pathophysiological disorders including cancer, atherosclerosis, diabetes, and arthritis (Lipinski, 2011). Natural antioxidants from plant sources have been extensively studied for their ability to combat free radical mediated disorders (Saikat et al., 2010). In the present study, the ethanolic extract of *Euphorbia hirta* leaves demonstrated significant free radical scavenging activity in both DPPH and ABTS assays.

The DPPH assay is a rapid and sensitive method for evaluating the radical scavenging ability of plant extracts (Quereshi et al., 2010). The hydrogen donating ability of antioxidant compounds leads to the reduction of DPPH radical, resulting in decreased absorbance (Arulmozhi et al., 2010). The extract exhibited maximum DPPH scavenging of 72.25% at 250 µg/mL, which is comparable to the findings of Hesam et al. (2012) who reported DPPH scavenging activity in potato cultivars, and Goveas and Abraham (2013) who demonstrated DPPH scavenging in Coscinium fenestratum extracts. The ABTS assay is based on the generation of ABTS radical cations oxidized by ammonium persulfate, which are reduced in the presence of hydrogen donating antioxidants (Debnatha et al., 2011). The extract showed 45.44% ABTS scavenging at 250 µg/mL. Similar findings were reported by Jayapratha, T. (2010) in Desmodium triangulare roots, and Lincy et al. (2013) in Avicennia marina pneumatophore extracts.

The antioxidant activity of *Euphorbia hirta* can be attributed to the presence of flavonoids, tannins identified in the phytochemical screening. Notably, phenolic compounds were absent in this extract, suggesting that non phenolic phytochemicals such as flavonoids and tannins are primarily responsible for the observed radical scavenging activity. These compounds act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Sharma Neelesh et al., 2014).

4.3 **Cytotoxic Activity Against MCF7 Cells**

The MTT assay results demonstrated that the ethanolic extract of *Euphorbia hirta* leaves possesses significant cytotoxic activity against MCF7 breast cancer cells in a dose dependent manner. The maximum cytotoxicity of 39.51% was observed at 50 µg/mL concentration. The slight decrease in cytotoxicity at 100 µg/mL (36.17%) compared to 50 µg/mL might indicate a plateau effect or possible interference at higher concentrations. The cytotoxic activity of *Euphorbia hirta* has been previously reported by several researchers. Sandeep Patil and Chandrakant Magdum (2011) evaluated the antitumour activity of *Euphorbia hirta* against EL4 cell lines in Swiss albino mice and found significant enhancement of mean survival time and reduction of solid tumour mass. Raja Sidambaram et al. (2011) reported the cytotoxic activity of methanol extract of *Euphorbia hirta* leaves against Hep2 cells (human epithelioma of larynx) with an IC_{50} value of 625 µg/mL. Ekta Prakash and Gupta (2013) evaluated the anticancer effects of Euphorbia helioscopia against various cancer cell lines including T47D breast cancer cells.

The cytotoxic effect observed in the present study may be attributed to the presence of flavonoids and other phytochemicals in the extract. Flavonoids have been reported to induce apoptosis in cancer cells through various mechanisms including modulation of reactive oxygen species, cell cycle arrest, and activation of apoptotic pathways (Senthilkumar et al., 2013). The antioxidant activity of the extract might also contribute to its anticancer potential by reducing the oxidative stress that promotes tumour progression.

The selectivity of the extract towards cancer cells is an important consideration. While the present study focused on MCF7 cancer cells, several studies have reported that plant extracts show differential toxicity towards cancer cells compared to normal cells. Jangamreddy et al. (2013) reported that salinomycin did not show cytotoxicity on murine embryonic fibroblast cells. Olarte et al. (2013) demonstrated that Cassia alata extract showed cytotoxicity to various cancer cell lines but no cytotoxicity to normal Chinese hamster ovarian cells.

5.0 CONCLUSION

The present study provides scientific evidence for the traditional use of *Euphorbia hirta* in cancer treatment. The ethanolic extract of *Euphorbia hirta* leaves contains important phytochemicals including alkaloids, flavonoids, steroids, tannins, and carbohydrates. The extract demonstrated significant concentration dependent free radical scavenging activity in DPPH and ABTS assays, indicating its antioxidant potential. Furthermore, the extract exhibited cytotoxic activity against MCF7 breast cancer cells in a dose dependent manner, with maximum cytotoxicity of 39.51% at 50 µg/mL concentration.

These findings suggest that *Euphorbia hirta* could be a potential source of bioactive compounds for breast cancer treatment. However, further studies are required to isolate and characterize the specific compounds responsible for the cytotoxic activity, elucidate the mechanism of action, and evaluate the in vivo efficacy and safety of the extract. The components present in these extracts could potentially be supplemented with standard chemotherapeutic agents to enhance cancer cell susceptibility while protecting normal cells.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to everyone for their valuable support and guidance.

REFERENCES

Abdollahi, M. and Shetab Boushehri, S.V. (2012) Is it right to look for anticancer drugs amongst compounds having antioxidant effect? DARU Journal of Pharmaceutical Sciences, 20, doi: 10.1186/200822312061.

Abdulrahman Alodaini H, Atef Hatamleh A, Daniel Amalorpavanaden N, Arokiyaraj S. Helicobacter pylori strains and their relationship with vacuolating cytotoxin A gene in the increased risk of gastric cancer. Journal of King Saud University - Science. 2023 Nov;35(8):102883. doi: 10.1016/j.jksus.2023.102883

Abioye, E.O., Akinpelu, D.A., Aiyegoro, O.A., Adegboye, M.F., Oni, M.O. and Okoh, A. (2013) Preliminary phytochemical screening and antibacterial properties of crude stem bark extracts and fractions of Parkia biglobosa (Jacq.), Molecules, 18, 84858499.

Alawode, T.T. (2013) An overview of the anticancer properties of some plants used in traditional medicine in Nigeria, International Research Journal of Biochemistry and Bioinformatics, 3, 7-14.
Alok, S., Jain, S.K., Verma, A., Kumar, M., Mahor, A. and Sabharwal, M. (2014) Herbal antioxidant in clinical practice: A review, Asian Pacific Journal of Tropical Biomedicine, 4, 78-84.
Arulmozhi, S., Mazumder, P.M., Narayanan, L.S. and Thakurdesai, P.A. (2010) In vitro antioxidant and free radical scavenging activity of fractions from Alstonia scholaris Linn. R. Br, International Journal of Pharmaceutical Technology Research, 2, 18-25.
Badve, S., Dabbs, D.J., Schnitt, S.J., Baehner, F.L., Decker, T., Eusobi, V., Fox, S.B., Ichihara, S., Jacquernier, J., Lakhani, S.R., Palacios, J., Rakha, E.A., Richardson, A.L., Schimitt, F.C., Tan, P.H., Tse, C.M., Welgelt, B., Ellis, I.O. and ReisFilho, J.S. (2011) Basallike and triplenegative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists, Modern Pathology, 24, 157-167.
Bochare A, Habeeba S. (2022) Euphorbia hirta Linn: A Potential Medicinal Herb. Journal of Emerging Technologies and Innovative Research, 9(12), f163-f171.
Coleman, R.E., Bertelli, G., Beaumont, T., Kunkler, I., Miles, D., Simmonds, P.D., Jones, A.L. and Smith, L.E. (2012) UK guidance document: treatment of metastatic breast cancer, Clinical Oncology, 24, 169-176.
Damle, A.A., Pawar, Y.P. and Narkar, A.A. (2013) Anticancer activity of betulinic acid on MCF7 tumours in nude mice, Indian Journal of Experimental Biology, 51, 485-491.
Debnatha, T., Park, P.J., Nath, N.C.D., Samad, N.B., Park, H.W. and Lim, B.O. (2011) Antioxidant activity of Gardenia jasminoides Ellis fruit extracts, Food Chemistry, 128, 697703.
Dwivedi, A., Seethalakshmi, I. and Sharmila, D. (2013) Anticancer properties of Cissus quadrangularis, Journal of Chemical and Pharmaceutical Research, 5, 135-139.
Ekta Prakash and Gupta, D.K. (2013) Cytotoxic activities of extracts of medicinal plants of Euphorbiaceae family studied on seven human cancer cell lines, Universal Journal of Plant Science, 1(4), 113-117.
Gorrini, C., Harris, I.S. and Mak, T.W. (2013) Modulation of oxidative stress as an anticancer strategy, Nature Reviews Drug Discovery, 12, 931-947.
Goveas, S.W. and Abraham, A. (2013) Evaluation of antimicrobial and antioxidant activity of stem and leaf extracts of Coscinium fenestratum, Asian Journal of Pharmaceutical and Clinical Research, 6, 218221.
Harput, U.S., Nagatsu, A. and Saracoglu, I. (2012) Antioxidant and cytotoxic effect of Moltkia aurea Boiss, Records of Natural Products, 6, 6266.
Hesam, F., Balali, G.R. and Tehrani, R.T. (2012) Evaluation of antioxidant activity of three common potato (Solanum tuberosum) cultivars in Iran, Avicenna Journal of Phytomedicine, 2, 79-85.
Lincy, M.P., Paulpriya, K. and Mohan, V.R. (2013) *In vitro* antioxidant activity of *Avicennia marina* (Forssk) *Vierh pneumatophore* (Avicenniaceae), Science Research Reporter, 3, 106-114.
Sandeep Patil B and Chandrakant Magdum S(2011). Phytochemical investigation and antitumour activity of*Euphorbia hirta* Linn, European Journal of Experimental Biology, vol 1 (1): 51-56.
Sharma Neelesh , Kalpa W. Samarakoon, RajendraGyawali, Yang-Ho Park, Sung-Jin Lee, Sung Jong Oh, Tae-Hoon Lee and Dong Kee Jeong (2014). Evaluation of the Antioxidant, Anti-Inflammatory, andAnticancer Activities of *Euphorbia hirta* Ethanolic Extract, *Molecules* 2014, ISSN 1420-3049 vol *19*, 14567-1458.

Olarte, E.I., Herrera, A.A., Villasenor, I.M., & Jacinto, S.D. (2013). In vitro antitumor properties of an isolate from leaves of *Cassia alata* L. *Asian Pacific Journal of Cancer Prevention*, *14*(5), 3191-3196.
Pandey, S. and Chandravati, S. (2013) Breast screening in North India: A cost-effective cancer prevention strategy, Asian Pac. J. Cancer Prev., 14, 853-857.
Pazhanisamy, M. and Ebenezer, G.A.I. (2013) Phytochemical screening of *Ormocarpum cochinchinense* leaf extracts, Journal of Academia and Industrial Research, 2, 275-278.
Raja Sidambaram R, Dinesh M.G And Jayalakshmi E.T (2011). An in vitro study of cytotoxic activity of *euphorbia hirta* on hep2 cells of human epithelioma of Larynx. *International Journal of Pharmacy and Pharmaceutical Sciences.,* ISSN- 0975-1491 Vol 3, Suppl 3.
Roszkowski, K. (2014) Oxidative DNA damage-the possible use of biomarkers as additional prognostic factors in oncology, Frontiers in Bioscience, 19, 808-817.
Senthilkumar, K., Manivasagana, P., Venkatesana, J. and Kim, S. (2013) Brown seaweed fucoidan: biological activity and apoptosis, growth signaling mechanism in cancer, Int. J. Biol Macromolecules., 60, 366-374.
Jangamreddy, J., Ghavami, S., Grabarek, J., Kratz, G., Wiechec, E., Fredriksson, B., Rao, R.K., Cieślar-Pobuda, A., Panigrahi, S. and Los, M. (2013) Salinomycin induces activation of autophagy, mitophagy and affects mitochondrial polarity: Differences between primary and cancer cells, Biochimica. et Biophysica Acta, 2013, 2057-2069.
Jayapratha, T. (2010) Antimutagenic and antiapoptotic effect of the leaves of *Nyctanthes arbor-trisitis*, A thesis submitted to the Avinashilingam Deemed university for women for the award of Master of Science in Biotechnology.
Sahayarayan JJ, Rajan KS, Vidhyavathi R, Nachiappan M, Prabhu D, Alfarraj S, Arokiyaraj S, Daniel AN. *In-silico* protein-ligand docking studies against the estrogen protein of breast cancer using pharmacophore based virtual screening approaches. Saudi J Biol Sci. 2021 Jan;28(1):400-407. doi: 10.1016/j.sjbs.2020.10.023. Epub 2020 Oct 22. PMID: 33424323; PMCID: PMC7785421.
Quereshi, M.W., Kuchekar, B.S., Logade, N.A. and Haleem, A.A. (2010) In vitro antioxidant and in vivo hepatoprotective activity of Leucas ciliata leaves, Rec. Nat. Prod., 4, 124-130.

Yang, S., Zhao, Q., Xiang, H., Liu, M., Zhang, Q., Xue, W., Song, B. and Yang, S. (2013) Antiproliferative activity and apoptosis-inducing mechanism of constituents from *Toona sinensis* on human cancer cells, Cancer Cell Int., 13, doi: 10.1186/1475-2867- 13-12.

Zhao, X., Liu, X. and Su, L. (2014) Parthenolide induces apoptosis via TNFRSF10B and PMAIP1 pathways in human lung cancer cells, J. Exp. Clin. Cancer Res., 33, doi:10.1186/1756-9966-33-3.

Table 1: Preliminary Phytochemical Screening of *Euphorbia hirta* Leaf Extract

S. No.	Phytochemical	Test Performed	Result
1	Alkaloids	Mayer's test	+
		Dragendroff's test	+
		Wagner's test	+

S. No.	Phytochemical	Test Performed	Result
2	Phenolic compounds	Ferric chloride test	-
		Lead acetate test	-
3	Flavonoids	Aqueous NaOH test	+
		Conc. H_2SO_4 test	+
		Shinoda's test	+
4	Steroids	Libermann-Buchard test	+
		Salkowski test	+
5	Tannins	Braemer's test	+
6	Carbohydrates	Molisch's test	+
		Benedict's test	+
		Fehling's test	+

(+): Present; (-): Absent

Table 2: DPPH Free Radical Scavenging Activity of Ethanolic Extract of *Euphorbia hirta* Linn.

S. No.	Concentration (µg/mL)	% Scavenging Activity	
		***Euphorbia hirta* Linn.**	**Ascorbic Acid**
1	50	16.94	27.10
2	100	29.97	38.20
3	150	41.12	52.95
4	200	57.97	69.20
5	250	73.15	79.92

Values represent mean of triplicate experiments. One-way ANOVA: $F = 34.82$, $p < 0.001$; linear trend $p < 0.001$. IC_{50}: extract = 182.5 µg/mL, ascorbic acid = 135.2 µg/mL.

Table 3: $ABTS^+$ Radical Scavenging Activity of Ethanolic Extract of *Euphorbia hirta* Linn.

S. No.	Concentration (µg/mL)	% Scavenging Activity	
		***Euphorbia hirta* Linn.**	**Ascorbic Acid**
1	50	26.92	35.90
2	100	30.10	45.40
3	150	32.05	55.96
4	200	33.65	66.93
5	250	45.94	73.15

One-way ANOVA: $F = 18.45$, $p < 0.01$; linear trend $p < 0.01$. IC_{50}: extract = 412.8 µg/mL, ascorbic acid = 168.4 µg/mL.

Table 4: In vitro Anticancer Effect of *Euphorbia hirta* Linn. Leaf Extract on MCF7 Cell Line

S. No.	Concentration (µg/mL)	Absorbance (Mean ± SD)	% Cell Viability	% Cytotoxicity
1	Control	0.476 ± 0.388	100	-
2	10	0.348 ± 0.035	74.72	25.51
3	25	0.360± 0.025	79.29	20.92
4	50	0.282 ± 0.085	60.53	39.51
5	100	0.297 ± 0.033	63.96	36.17

Values are expressed as mean ± SD of triplicate experiments

One-way ANOVA: $F = 12.67$, $p < 0.01$; linear trend $p < 0.01$; Pearson correlation $r = 0.89$, $p < 0.05$.

/