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Invitro Anticancer Effect of D. Mespiliformis Stem Back Chloroform Extract Against Breast Cancer Cell Lines MCF-7

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ABSTRACT
Breast cancer is among the most prevalent cancers in Nigeria constituting aipproximately 27% of all new cases and representing a significant and growing public health concern. The impact of cancers continues to grow due to several factors, leading to an estimated mortality of up to 10 million annually. Medicinal plants have been utilized widely for treatment of diseases including cancer. This study evaluated the phytochemical analysis and cytotoxic effects of D. mespiliformis stem bark extract against human breast adenocarcinoma cells (MCF7). The extract was prepared using D. mespiliformis stem bark and chloroform as a solvent. The phytochemical constituents were identified using qualitative method. The anticancer effect was determined using cell titre Glo pro mega assay while total intracellular glutathione (GSH) was measured to assess whether redox imbalance contributed to the observed cytotoxic response. The phytochemical screening of the extract reveals the presence of important compounds. At 48 hours, the extract exhibited a strong cytotoxic effect (IC_{50} of 21.75 µg/mL) against MCF-7 cells while sparing MCF-10. Mechanistic assessment showed that the extract reduced intracellular GSH levels in MCF-7 cells, indicating that glutathione depletion and oxidative stress may be involved in the cytotoxic effect. The cytotoxicity of the extract might be attributed to the presence of important secondary metabolites. The findings from the present study highlights the chemopreventive potential of D. mespiliformis stem chloroform extract against MCF-7 breast Cancer cell. Further studies are required to isolate the active constituents, confirm the molecular mechanism and evaluate safety in broader experimental models.

Keywords:
Invitro Anticancer,
Mespiliformis,
Chloroform
MCF-7

INTRODUCTION

Cancer is a life-threatening disease characterized by the abnormal, uncontrolled growth and spread of malignant cells, which can invade surrounding tissues and metastasize to distant organs. It involves a broad group of diseases capable of affecting any part of the body and is a leading global health concern, causing millions of deaths annually due to complications from metastatic progression (WHO 2025; Brown et al., 2023; Ferlay et al., 2013).

More than 30% of cancers are caused by modifiable behavioral and environmental risk factors, including tobacco and alcohol use, dietary factors, insufficient regular consumption of fruit and vegetable, overweight and obesity, physical inactivity, chronic infections from Helicobacter pylori, hepatitis B virus, hepatitis C virus and some types of human papilloma virus, environmental and occupational risks including exposure to ionizing and nonionizing radiation (Balalau. et al., 2017).

Medicinal plants have a long history of usage in traditional medicine systems as sources of therapeutic remedies. They have served as valuable resources for the discovery and development of bioactive compounds with diverse pharmacological activities. The rich biodiversity of medicinal plants offers a wide array of chemical constituents that possess potential health benefits (Rahalison *et* al., 2012). Indigenous communities worldwide have long depend on the healing properties of medicinal plants, and their traditional knowledge has been instrumental in identifying and utilizing bioactive compounds (Heinrich *et* al 2020). The bioactive substances present in medicinal plants include alkaloids, flavonoids, terpenoids, phenolics, essential oils, among several others which are commonly known as Phytochemicals. They are chemicals produced by plants through primary or secondary metabolism (Molyneux *et* al., 2007). They generally have biological activity in the plant host and play a role in plant growth,

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defense against competitors, pathogens, or predators (Molyneux *et* al., 2007). These substance's pharmacological characteristics, such as their antibacterial, antioxidant, anti-inflammatory, anticancer, and immunomodulatory actions, have been thoroughly investigated (Zengin et al., 2020 and Rahalison *et* al., 2012). *Diospyros mespiliformis* (commonly referred to as African Ebony or jackal berry) is an evergreen tree with a rounded dense crown, belonging to the family of Ebenaceae that can grow successfully across many regions of Africa, including Nigeria (leonard 1957). it varies considerably in height, sometimes flowering as a shrub when just 3 meters tall, growing 12 - 15 meters tall in drier areas while up to 25 meters or more in the wetter areas (von Maydell 1990). The female tree bears fruit in the dry season and these are eaten by many wild animals; they are oval-shaped, yellow or purple when ripe and about 20–30mm in diameter. The fruit remain embedded in the persistent calyx lobes (Hyde *et al.,* 2018*).* According to National Research Council *(2008)*, the fruits of the plant is a traditional food in Africa and has a potential to improve nutrition, boost food security, foster rural development and support sustainable land care. The leaves, bark and roots of the tree contain tannin, which can be used as a styptic to staunch bleeding. The roots are consumed to purge parasites and are thought to be a remedy for leprosy. *D. mespiliformis extracts* were reported to have a variety of pharmacological activities, including antimicrobial, antiproliferative, antiparasitic, antioxidant, anti-inflammatory, antiviral, anti-hypersensitivity, antidiabetic (Mustapha *et al., 2022 &* Vandi *et al., 2022*) and anticancer (Sani *et al.,* 2026) properties. This study aimed to uncover qualitatively, the phytochemical composition of D. *mespiliformis* stem bark chloroform extract and its anticancer effect against human breast cancer cell lines (MCF-7).

MATERIALS AND METHODS

Selection and Collection of Medicinal Plants

Fresh leaves and stem bark of *D. mespiliformis* were collected around Dandume Local Government Area of Katsina State, Nigeria **(11^{O} 25'17" N, 7^{O} 9' 7" E)**. The sample was identified by a professional botanist in the Department of Biology, faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University Katsina, Nigeria.

Sample preparation and extraction

The collected samples were washed with ordinary tap water then air dried at room temperature and grinded into a fine powdered form using manual grinder. Twenty grams of the grinded samples were macerated in 200ml of the solvent (aqueous and chloroform). The solutions were kept overnight on a rotary shaker at 150rpm at 25^{O}C. The mixtures were filtered and concentrated to dryness using evaporator as described by Upreti et al., (2018). The crude extracts were kept in airtight glass vials and stored at 4°C until use.

Qualitative Phytochemical screening

Alkaloids

Wagner's test: Six drops of Wagner's reagent was added to 2 mL of the crude extract. The Wagner's reagent was prepared by mixing iodine in potassium iodide solution. The formation of brown or reddish precipitate indicates the presence of alkaloids (Arya *et al*., 2012).

Flavonoids

Ferric chloride test: 2ml of the crude extract was treated with a few drops of ferric chloride solution. The intense green colour of the solution shows the presence of flavonoids (Pooja and Vidyasagar 2006).

Steroids

Salkowski test: About 100 mg of dried crude extract was dissolved in 2 mL of chloroform. 3 drops of sulphuric acid was added carefully to the extract to form a lower layer. A reddish-brown colour at the interface indicate the presence of steroidal ring (Iqbal *et al*., 2015).

Glycosides

Keller-Killani test: 5ml of the extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid. A brown ring at the interface indicate the presence of cardiac glycosides (Shanmugam et al., 2010).

Terpenoids

Salkowski test: 1mg of the extract was mixed with 2ml chloroform and 1ml concentrated sulphuric acid. The formation of reddish-brown colour at the interface indicates the presence of terpenoids (Iqbal *et al*., 2015).

Phenols

Liebermann's test: To 1ml of extract, 1ml of sodium nitrite, few drops of diluted sulphuric acid and 2ml of diluted NaOH were added. Appearance of deep red or green or blue colour indicates presence of phenol (Shanmugam et al., 2010).

Tannins

Modified Prussian blue test: 1ml of 0.008M potassium ferricyanide followed by 1ml of 0.02M FeCl3 in 0.1M HCl were added to 1ml of the extract. Appearance of blue colour indicates the presence of tannins (Shanmugam et al., 2010).

Saponins

Foam test: 2ml of distilled water was added to 2 mL of crude extract. The mixture was shaken vigorously for 15 minutes period. The persistence of foam produced after

15 minutes indicates the presence of saponins (Rao *et al.*, 2016).

Mammalian tissue culture

Measures such as authentication of cell line, cryopreservation, and avoidance of cross-contamination of cell lines were all taken. Furthermore, the cells were checked for microbial contamination (regular mycoplasma test).

Cell lines

For the present study, the media was supplemented with filter sterilized 10% foetal bovine serum (FBS), 2mM glutamine, 1mM sodium pyruvate, 100 µg/mL streptomycin and 100 U/mL penicillin all purchased from Invitrogen UK. The breast cancer cells were maintained at 37ºC in a tissue culture incubator with an atmosphere containing 5% CO_2.

Sub-culturing the cells

The breast cancer cells used this study were Sub-cultured regularly to ensure their survival, regular supply for the study and maintain proper growth conditions. It was routinely done with cells that reached 80-90% confluence either in flask or tissue culture plates. All the procedures were done using sterile techniques performed in a sterile tissue culture hood. Briefly, in the process of sub-culturing, the media, buffers and trypsin were placed in water bath at 37°C for at least 30 min prior to sub culturing the cells. Tissue culture lab coat and gloves were worn. The gloves were sprayed with 70% ethanol. The laminar flow in the hood was turned and the working surface of tissue culture hood was swabbed with 70% ethanol. Any apparatus, media, trypsin and buffers taken inside the hood was first swabbed by 70% ethanol except for tissue culture flasks and plates. The tissue culture flask needed to be sub-cultured (>80% cell confluence) was taken out of the incubator, observed under the microscope and placed into the hood. With the help of aspirator attached to the suction pump, the old medium was removed from the flask. 4mL of PBS (Invitrogen) was added to the cells and the flask gently swirled to wash away the old media and dead cells. PBS was aspirated out. 1 mL of pre-warmed 0.25% trypsin (Invitrogen) was gently added on top of cells using serological pipette, and the flask swirled to ensure spreading of trypsin on the entire surface area of the flask. The flask was taken into tissue culture incubator maintained at 37ºC with 5% CO_2 atmosphere and incubated for 5 min (or until the cells were detached as observed under the light microscope). After the cells detached, 4 mL of pre-warmed cell culture media was added to the flask to stop the further action of trypsin and dilute the cells. Using serological pipette, the cell suspension was mixed thoroughly by pipetting up and down to ensure total detachment and to break any cell clumps formed. After this, the number of cells in the suspension was determined and either seeded into other flasks for propagation and cell culture maintenance, or cryo-frozen for future use (Kankia et al., 2021).

Cell treatments extracts

The extract used in this research work involved the preparation of stock solution of D. *mespiliformis* leaf aqueous extracts and stored. Working concentrations (50, 100, 250, 500 and 1000µg/ml) were achieved by diluting the stock in the media used and were based on prior literature. Prior to treatment with these extracts, breast cancer cells (MCF-7) were taken out of the incubator; old media removed and pre-warmed PBS added. Pre-warmed media was then transferred to falcon tubes (corning) in the tissue culture hood and the required concentration was added to achieve the working concentrations. The PBS over the cells was taken out and the media with the extract solution carefully added. The amount of media added was dependent upon the type of tissue culture vial in which the cells were grown. For example, the cell lines were maintained in RPMI 1640 media (Gibco® Invitrogen) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 1 mM sodium pyruvate, 100 µg/ml streptomycin and 100 U/ml penicillin in an atmosphere of 5% CO2 and incubated at 37°C. Before experimental treatments, cells were grown for 24h in RPMI 1640 media prepared as above. All the extracts were used by directly diluting the extracts in media to desired final concentrations (Kankia et al., 2021).

Cell viability assay

The CellTiter-Glo® 2.0 assay kit (Promega) was used to determine cell viability, as described by the manufacturer. Briefly, cells were seeded in a luminometer compatible 96-well plate and allowed to adhere for 24h. Following treatments using different concentrations (50, 100, 250, 500 and 1000µg/ml) of our working extract, the plate and its contents were equilibrated to room temperature for approximately 30 min, a volume of CellTiter-Glo 2.0 reagent equal to the volume of cell culture medium present in each well the contents were then mixed for 2 min on an orbital shaker to induce cell lysis and the plate was then incubated at room temperature for 10 min to stabilize the luminescent signal and finally the luminescence was recorded using luminometer (MODULUS, Promega). The luminescent signal is proportional to the amount of ATP in the sample, which indicates the presence of metabolically active (Kankia et al., 2021).

Measurement of Total Glutathione (GSH)

The measurement of total GSH was performed using GSH/GSSG-Glo™ Assay (Promega) according to manufacturer's protocols. Briefly 1x104 cells where seeded in luminometer-compatible 96-well plates and

allow to grow overnight at 37°C in a 5% CO2 culture incubator. The following day, the cells were treated accordingly. Following this, the contents in the cell were removed. Then 50 µL per well of GSH Lysis Reagent, that was prepared no longer than 30 min before use, was added. The lysate was shaken at room temperature for 5 min on a plate shaker. Following this, 50 µL per well of Luciferin Generation Reagent was added to all wells. The Luciferin Generation Reagent was prepared within 30 min before use. The plates were then shaken briefly and incubated at room temperature for 30 min. Finally, 100 µL per well of Luciferin Detection Reagent was added and the plates were shaken briefly, waited for 15 min to stabilize the luminescent signal and finally the luminescence was recorded using luminometer (MODULUS, Promega) (Kankia et al., 2021).

STATISTICAL ANALYSIS

All quantitative data were expressed as the means ± SD (standard deviation). Statistical significance between the control and treated groups was determined by One-Way ANOVA using Graph Pad Prism 7 software.

RESULTS AND DISCUSSION

TABLE 1. Qualtatve phytochemical screening of D. *mespiliformis* stem bark chloroform extract.

Phytochemical screening of *D. mespiliformis* stem bark extract revealed the presence of alkaloids, steroids, glycosides, phenols and other important secondary metabolites while flavonoids, terpenoids and saponins were absent from the present study as presented in **Table 1** below**.**

Phytochemicals	Test	Inference	
Alkaloids	Wagner's test	Appearance of brown or reddish precipitate	+
Flavonoids	Ferric chloride test	Appearance of red coloration	-
Terpenoids	Salkowsky test	Formation of reddish brown color at the interface	-
Phenolic acids	Liebermann's test	Appearance of deep red or green color	+
Saponins	Foam test	Formation of persistent foam	-
Tannins	Modified Prussian blue test	Appearance of blue coloration	+
Glycosides	Keller-killani test	Appearance of a brown ring at the interface	+
Steroids	Salkowski test	Presence of a reddih-brown color at the interface	+

KEY: + = presence, - = absence

Effect of D. *Mespiliformis* stem bark chloroform Extract of on Normal Breast Epithelial Cells.

Normal Human breast cells were treated with different concentrations of *D. mespiliformis* stem bark chloroform extract and incubated for 24, 48, and 72 hours. Cell viability was determined using the CellTiter-Glo® 2.0 Assay Kit (Promega).

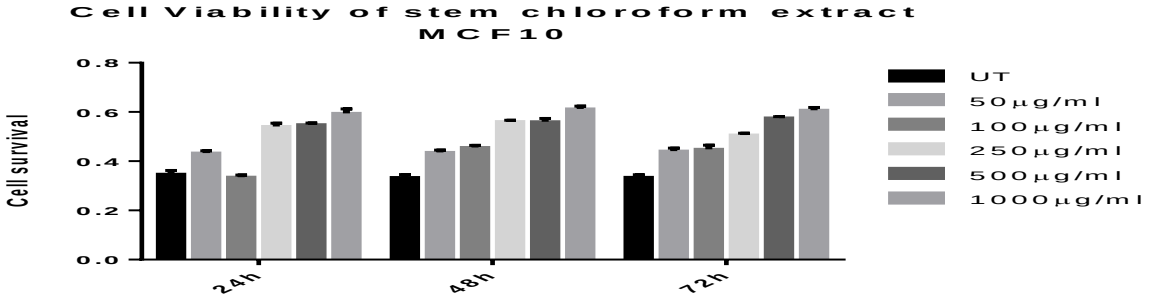

Figure 1: Cell viability assay: D. *mespiliformis* stem bark chloroform extract against normal breast cells (MCF-10).

Treatment of MCF-10A cells with different concentrations of the extract at varying incubation periods showed minimal cytotoxicity. Cell viability in the treated groups remained higher than that of the untreated control (UT), indicating low toxicity toward normal breast epithelial cells. Furthermore, cell viability increased with increasing extract concentration, suggesting that the extract may contain bioactive constituents that support the survival and proliferation of MCF-10A cells

Effect of D. *Mespiliformis* stem bark chloroform Extract of against Breast Cells (MCF7)

The cell viability of *D. Mespiliformis* stem bark chloroform extract shows exceptional potency specifically at 48hours incubation with an IC_{50} of 21.75µg/mL while the effect is less sustained after 72hours incubation as presented in **Figure 2** below.

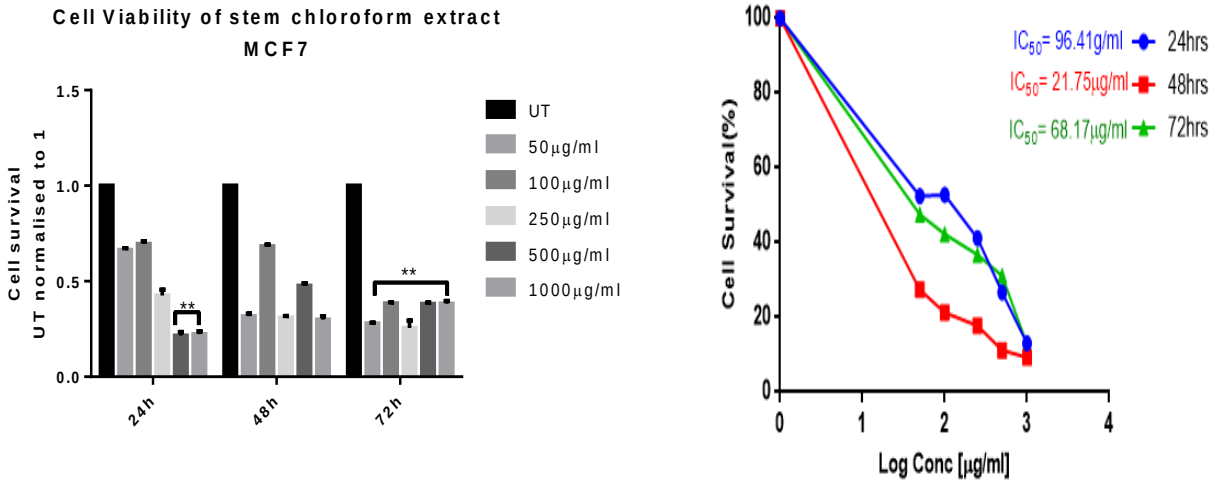

Figure 2: Cell viability assay: Breast cancer cells (MCF-7) treatment with different concentrations of D. *mespiliformis* stem bark chloroform extract.

Total Glutathione Measurement for Normal Breast Cells and Breast Cancer Cells

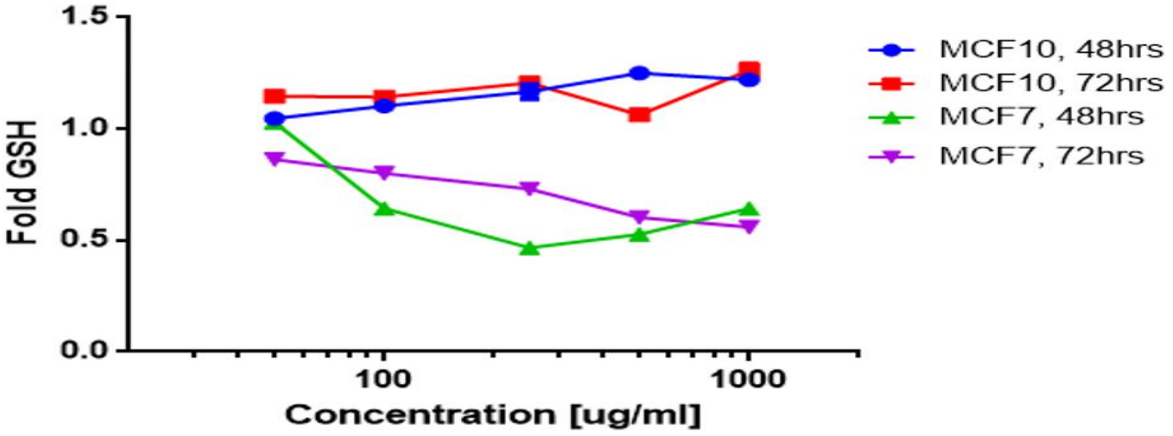

Figure 3. Measurement of total GSH for MCF-10 and MCF-7 treated with different concentrations of D. *mespiliformis* stem bark chloroform extract at varying incubation periods: Increasing extract concentration was associated with altered cellular glutathione levels, with more pronounced GSH depletion in MCF-7 cells than in MCF-10 cells

One of the major successes in phytochemistry is the interest it has arose in modern medicine. Phytochemical studies are used in many disciplines not only because of their results but also their advantages and hope they offer

in drug production in the pharmaceutical field and chemotherapy (Issa *et al.,* 2025). The phytochemical screening carried out in the present study reveal the presence of alkaloids, phenols, tannins, glycosides and steroids in the D. *mespiliformis* stem bark chloroform extracts while Flavonoids, Saponins and Terpenoids were absent as presented in **(TABLE 1)** above. The presence of alkaloids, steroids, tannins and absence of flavonoids and Saponins in this study supports the findings from different literatures previously (Ebbo *et al.,* 2022; Vandi *et al., 2022;* Hawas *et al.,* 2022), however they differ in some aspects as these authors also demonstrate the presence of Terpenoids which was found absent in the present sudy. Similarly, the presence of flavonoids and phenols as well as the absence of Saponins in this study were not in agreement with Shagal *et al., (2013),* who discovered the absence of flavonoids and phenols as well as the presence of Saponins in aqueous extract of *D. mespiliformis* stem bark While support their findings in respect to presence of alkaloids and tannins.

The presence of these bioactive compounds is significant because such metabolites are known to contribute directly to pharmacological properties of medicinal plants. For instance, alkaloids are well documented for their anticancer activities (Lu et *al.,* 2012), acting by interfering with DNA replication and mitosis. Tannins exhibit anticancer, antioxidant, and anti-inflammatory activities (Dhyani et al., 2022), while saponins can inhibit metastasis, angiogenesis, and multi-drug resistance by inducing apoptosis and modulating the immune system (Elekofehinti et al., 2021). This observation helps to explain why the aqueous leaf extract later produced stronger anticancer effects compared to the other extracts.

The cytotoxic effects of the extract in the present study was investigated using cell titre Glo assay Promega against human breast cancer cell line (MCF-7), and the results demonstrated that our extract is able to reduce cell growth in both dose and time-dependent manners. One of the major important finding in the anticancer activity of D. *mespiliformis* stem chloroform extract in the present study is the selective toxicity it exhibited in killing cancer cells. While the extract reduces the growth of MCF-7 breast cancer cells, it shows no effect on normal breast epithelial cells (MCF-10) even at the highest extract concentration and longest incubation period of 72hours as presented in (**figure 1**) above. This shows that, the bioactive compounds present in D. *Mespiliformis* the stem chloroform extract can disguise between cancerous and non-cancerous cells and is important as it can helps in minimizing severe side effects. The ability of this extract in the present study to target only cancer cells while sparing normal cells supports the possibility of producing good anticancer agents from this plant.

The extract presented a unique profile in inhibiting the growth of breast cancer cells (MCF-7). At 48 hours, the extract showed a strong cytotoxic effect with an IC_{50} of 21.75 µg/mL, but loses its potency 72 hours incubation (**figure 2**). This unusual model might be explained by either the presence of compounds that act quickly but are unstable in long incubation period, surviving cells induces a resistance to the effect of the extract after 48 hours or possible degradation of active compounds over time in the culture environment. This observation is very interesting because it highlights the complications of natural plant extracts, where many compounds with different stabilities and targets combine to produce biological responses.

Moreover, the present study didn't limited to the anticancer activity of D. *Mespiliformis* the stem chloroform extract alone, rather, It goes further to explain the possible anticancer mechanism of action of this extract by measuring the total intracellular glutathione level of the cells after treatment with different concentrations of the extract solution and incubation at varying period. Glutathione is one of the most important non-enzymatic antioxidants in cells and it plays an important role in scavenging of free radicals and maintaining redox balance. The results demonstrated that *D. mespiliformis* stem chloroform extract reduced the total glutathione level in MCF-7 cells. The depletion of this glutathione level was more pronounced in cancer cells than in the normal MCF-10 cells as shown in **figure 3.** Above. This finding support Sani et al., (2026) who discovered that, the treatment of MCF-7 breast cancer cells with different concentratios of *D. Mespiliformis* leaf aqueous extract solution courses the depletion of total intracellular glutathione level.

CONCLUSION

Generally, this study has shown that *Diospyros mespiliformis* stem bark possess some important phytochemicals such as alkaloids, steroids, tannins and others which might be highly responsible for its anticancer properties. The stem chloroform extract also appeared to be selectively toxic as it inhibit breast cancer cell growth while sparing normal cells. The depletion of intracellular glutathione in cancer cells provides a mechanistic basis for this selective cytotoxicity, supporting the view that the extracts induce a lethal oxidative stress to the breast cancer cells thereby, inhibiting their growth. This result is important as it support the traditional use of D. mespiliformis in herbal medicine and open new opportunities for drug discovery.

REFERENCE

Arya, V., Thakur, N. and Kashyap, C. P. (2012). Preliminary phytochemical analysis of the extracts of Psidium leaves. Journal of Pharmacognosy and Phytochemistry, 1(1):15.

Balalau D.O., Sima R.M., Bacalbasa N., Banu P., Bălălău C., Ples L., Stanescu A.D. (2017) High-grade cervical dysplasia in pregnancy–psychological and medical challenges. J. Mind Med. Sci.; 4:2430.doi: 10.22543/7674.41.P2430.

Brown, J. S., Amend, S. R., Austin, R. H., Gatenby, R. A., Hammarlund, E. U., and Pienta, K. J. (2023). Updating the definition of cancer. Mol. cancer Res. MCR 21, 1142–1147. doi:10.1158/1541-7786.MCR-23-0411

Dhyani P, Quispe C, Sharma E, Bahukhandi A, Sati P, Attari D.C.(2022). Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine,vincristine, vindersine, vinorelbine and vincamine. Cancer cell int. 22,206. doi:10.1186/s12935-022-02624-9.

Ebbo A.A., Sani D., Suleiman M.M., Ahmad A., Hassan A.Z. (2022). Assessment of antioxidant and wound healing activity of the crude methanolic extract of Diospyros mespiliformis Hochst ex a. Dc (Ebenaceae) and its fractions in Wistar rats. S. Afr. J. Bot. 150:305–312. doi: 10.1016/ j. sajb.2022.07.034.

Elekofehinti O.O, Iwaloye O, Olawale F, Ariyo E.O. (2021). Saponins in cancer treatment: current progress and future prospects. Pathophysiol.official J. Int.Soc.pathophysiol. 28,250-272. Doi: 10.3390/pathophysiology28020017.

Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. (2013). Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. European journal of cancer. 49 (6):1374-403.

Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M. (2020). Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research on Cancer; (https:// gco.iarc.fr/today, accessed February 2021).

Hawas U.W., El-Ansari M.A., El-Hagrassi A.M. (2022). A new acylated flavone glycoside, in vitro antioxidant and antimicrobial activities from Saudi Diospyros mespiliformis Hochst. Ex A. DC (Ebenaceae) leaves. ZNC.77:387–393. doi: 10.1515/znc-2021-0291

Heinrich M, Leonti M, Nebel S. (2020). Ethnopharmacology and drug discovery. Journal of Ethnopharmacology. 251.112530.

Hyde, M.A. (2018). "Diospyros mespiliformis Hochst. Ex A. DC". *Flora of Zimbabwe*. Retrieved 20 November 2018.

Iqbal, E., Salim, K. A. and Lim, L. B. L. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (airy shaw) from Brunei Darussalam. Journal of King Saud University – Science, 27: 224-232.

Issa S, Ismaila B.D, Moussa K, Maello E, Aissatou A.G. (2025). Diospyrus mespiliformis hochst ex a. dc. Ethno-botanical study and therapeutic value. Journal of pharmacognosy and phytochemistry. 14(2):121-136.

Kankia, I. H., Paramasivan, P., Elcombe, M., Langdon, S. P., & Deeni, Y. Y. (2021). Nuclear factor erythroid 2-related factor 2 modulates HER4 receptor in ovarian cancer cells to influence their sensitivity to tyrosine kinase inhibitors. Exploration of Targeted Anti-tumor Therapy, 2(2), 187–203. https://doi.org/10.37349/etat.2021.00040

Leonard, J (1957). Ebenaceae. Flore du congo belge et du Ruanda-urundi. Spermatophytes (vol.3). jardin botanique national de Belgique.

Lu J.J, Bao J.L, Chen X.P, Huang M, Wang Y.T. (2012). Alkaloids isolated from natural herbs as an anticar agents. Evidence-based complementary altern. Med. 2012, 485042. Doi:10.1155/2012/485042.

Molyneux, RJ; Lee, ST; Gardner, DR; Panter, KE; James, LF (2007). Phytochemicals: the good, the bad and the ugly? Phytochemistry. 68 (22–24): 2973–85. Bibcode : 2007PChem..68.2973M. doi: 10.1016/ j.phytochem.2007.09.004. PMID 17950388. Archived from the original on 2023-07-03. Retrieved 2017-02-10.

Mustapha M.D., L.G. Hassan, K. J. Umar, K. Abubakar2, H.E. Mshelia3 A.J. Yusuf (2022). "Phytochemical screening and cytotoxic effects of Diospyros mespiliformis Hochst (Ebenaceae) root bark extract using Brine Shrimp (Artemia salina) Test". AJBAR Vol 1(6): 1-7, ISSN: 2811-2881

National Research Council (2008-01-25). "Ebony". Lost Crops of Africa: Volume III: Fruits. Vol. 3. National Academies Press. ISBN 978-0-309-10596-5. Retrieved 2008-07-25.

Pooja, S and Vidyasagar, G. M. (2016). Phytochemical screening for secondary metabolites of Opuntia dilleniihaw. Journal of Medicinal Plants Studies, 4(5): 39-43.

Rahalison L, Hamburger M, Hostettmann K. (2012). "Antimicrobial and antiparasitic activities of medicinal plants from Madagascar". In Ethnobotany of Madagascar. 389-404. CRC Press.

Rao, U. S. M., Abdurrazak, M. and Mohd, K. S. (2016). Phytochemical screening, total flavonoid and phenolic content assays of various solvent extracts of tepal of Musa paradisiaca. Malaysian Journal of Analytical Sciences, 20(5): 1181-1190.

Sani, J., Kankia, I. H., Nasir, A., Usman, A., Bawa-Sani, H. G., Usman, M. M., & Yar'adua, A. I. (2026). D. mespiliformis Leaf Aqueous Extract Exhibits Selective Anticancer Effects against Breast Cancer Cells Via Glutathione Depletion. Asian Journal of Biochemistry, Genetics and Molecular Biology, 18(8), 1–9. https://doi.org/10.9734/ajbgmb/2026/v18i8557

Shagal M. H, D. Kubmarawa, and H. Alim (2012)."Preliminary phytochemical investigation and antimicrobial evaluation of roots, stem-bark and leaves extracts of Diospyros mespiliformis" International Research Journal of Biochemistry and Bioinformatics (ISSN-2250-9941) Vol. 2(1) pp.011-015 January, 2012 Available online http://www.interesjournals.org/IRJBB

Shanmugam S, Sathish Kumar T and Panneer Selvam K, 2010. Laboratory handbook on Biochemistry.. PHI learning private limited Delhi.

Upreti A, Byanju B, Fuyal M, Chhetri A, Pandey P (2018) Evaluation of aamylase, lipase inhibition and in-vivo pharmacological activities of Eucalyptus camaladulensis Dehnh leaf extract. J Trad Complementary Med 30:1e7 https://doi.org/https://doi.org/10.1016/j.jtcme.2018.07.001

Vandi V.L., Amang A.P., Mezui C., Siwe G.T., Ndji G.L., Mbida H., Baponwa O., Tan P.V. (2022). Antihistaminergic and anticholinergic properties of the root bark aqueous extract of *Diospyros mespiliformis* (Ebenaceae) on hypersecretion of gastric acid induced in wistar rats. *eCAM.* 2022:5190499. doi: 10.1155/2022/5190499.

Von Maydell H. (1990). 'Trees and shrubs of the sahel. Their characteristics and uses'. Gesellschaft fur technische zusammenarbeit; Germany. ISBN 3-8236-1198-4

WHO (2025)."Cancer". Www.who.int. 3 February 2025. Retrieved 26 July 2025.

World Health Organization. (2025)."Cancer" Available at https://www.who.int/news-room/fact-sheets/detail/cancer

Zengin G, Sarikurkcu C, Aktumsek A. (*2020). "*Phenolic constituents, antioxidant and enzyme inhibitory activities of *Cruciata taurica eendermc to Turkey". Chemistry & Biodiersity. 2020; 17(2):e1900506*