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ORIGINAL ARTICLE

Cytotoxic Effect of Kigelia Africana Plant Extracts on Liver Cancer Cells

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ABSTRACT

Background: Various medicinal plants have much efficacious value as treatment of many fatal conditions including cancer. *Kigelia africana* has known therapeutic efficacy in different ailments and has been used as traditional medicine since ages.
Aim: To evaluate anticancer property of any drug or plant extract including HepG2 cell line.
Methodology: Anticancer activity of n-hexane and ethanolic extracts of *Kigelia africana* was checked. IC50 was evaluated via MTT assay, crystal violet assay was performed to check the viability of cells and trypan blue assay to count dead cells. Furthermore, muse analysis was performed using count and viability kit to count total living as well non-living cells.
Results: Cancer cells of HepG2 cell line showed reduced viability and proliferation with increased apoptosis when treated with *Kigelia* extracts.
Conclusion: Many drugs have been proved as anticancer but with severe side effects. Thus, phytoextracts have been tested in this study to evaluate their anti-cancer activity so minimize the side effect. *Kigelia africana* extracts were found to be effective against liver cancer cells.
Keyword: Kigelia africana, HepG2, MTT, liver cancer, anticancer drug, plant based drugs.

INTRODUCTION

Native knowledge of herbal medicine is a big source of the modern knowledge[1]. Historically, herbal drugs were used as poultices, tinctures, teas and powders followed by inventions and lastly as pure compounds. Medicinal plants have many medicinal activities and are used in herbalism. From many generations, this knowledge was transferred in families, cultures and tribes and is back bone of traditional medicine, as more than 3 billion people use traditional medicine daily in less developed countries[2]. *Kigelia africana (K. africana)* (Lam), belongs to the Bignoniaceae, is usually mentioned as sausage or *Magnolia acuminate* due to its huge sausage or cucumber-like fruit. It is referred to as "Nufutene" in local Asante-Twi in Ghana. *K. africana* grows throughout tropical Africa and wet savannah[3] in watercourses, open woodland, riverine fringes alluvial, shrubs, rain forests and high rainfall savanna. Loamy clay soils and damp or peaty rocks are also habitats from water level up to zoom altitude[4].

Hepatocellular carcinoma (HCC) often occurs in liver diseases at chronic stage. Its onset becomes worse due to late diagnosis or poor treatment of underlying hepatic disease. That's why, HCC is one of the leading causes of death due to cancer all over the world[5]. Less than 50% patients are cured by treatments such as surgury and transplantation and nearly 20% receive chemotherapy[6]. Other treatments include percutanous ethanol injection, radiofrequency ablation and transcatheter arterial chemoembolization[7-9] but at last stage, systemic chemotherapy is the only option as HCC is considered as chemotherapy-refractory cancer[10]. Molecular target therapy is also focused now as an alternate option for treatment of HCC as this technique can discriminate cancer cells from normal cells due to which, less normal cells are harmed. In this way, animal and human liver cell lines can be implicated for research purposes in order to understand phenomena of hepatocytes without keeping humans at risk directly[11-12]. Although, metabolic reactions of these hepatoma cell lines are more limited than normal liver cells, but still they are more beneficial in in-vitro studies, because they have stable phenotype (independent on donor availability), high availability rate, easy handling and unlimited mitotic activity. Out of all these hepatoma cell lines, HepG2 is considered most suitable for research purposes[13-14].

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MATERIALS AND METHODS

HEPG2 cell lines sampling: HepG2 cell line was obtained from animal cell and tissue culture laboratory of University of Lahore. The cell lines were kept in liquid nitrogen cylinder and then resuscitate from cryovials when there is need of culturing[15].
Culturing of hepg2 cell line: Cells were thawed and cultured in T75 flasks in Dulbecco's Modified Eagle's Medium (DMEM) (High Glucose) (Caisson's Lab, USA), supplemented with streptomycin (Caisson's Lab, USA) and penicillin (Caisson's Lab, USA) also 10% fetal bovine serum (FBS) (Sigma, Aldrich, USA) in humidified incubator at 5% CO_2 and 37°C temperature . Medium was replaced after every 2-3 days. For treatment, DMEM was used without FBS[16].
Cell viability analysis: Cell viability and cytotoxicity was evaluated in liver cancer cells between control and treated groups through MTT, crystal violet and trypan blue assays as mentioned in study of Maqbool T[15].
Muse analysis by *Kigelia Africana* plant extract treatment: Muse analysis was done according to protocol mentioned in study of Hadi et al[16].

RESULTS

MTT Assay of n-hexane and ethanolic extract of *Kgelia Africana:* Liver cancer cell line HepG2 was treated with n-hexane and ethanolic extracts of *K. Africana* at five different doses 0.1mg/ml, 0.2 mg/ml, 0.5 mg/ml, 1mg/ml and 2mg/ml for 72 hours. It was observed that there was a significantly less cytotoxic activity at the dose of 0.1mg/ml, and more cytotoxic activity at 2mg/ml of n-hexane extract as shown in table 1A and figure 1A. IC50 value was calculated at 1.47mg/ml as shown in figure 1B. There was a significantly less cytotoxic activity at the dose of 0.1mg/ml, and more cytotoxic activity at 1mg/ml of ethanolic extract as shown in table 1B and figure 2A IC50 value was calculated at 0.153mg/ml as shown in figure 2B.
Crystal violet assay of n-hexane and ethanolic extracts of *Kigelia Africana:* IC50 doses of n-hexane and ethanolic extracts of *K. Africana* were applied in crystal violet assay which is best assay for cell viability, where IC50 dose, 1.47mg/ml of n-hexane extract of *K. Africana* was applied for 72 hours. It showed significantly decrease in cell viability as shown in table 2A and figure 2A. In case of ethanolic extract, *K. Africana* decreased cell

viability after IC50 dose, 0.153mg/ml treatment of extract after 72 hours as shown in table 2B and figure 2B.

Table 1A: Cell viability values of untreated and treated hepg2 cells

Groups & Doses	Values(±SEM)
Untreated	1.06±0.0234
Treated with n-hexane extract of *K. africana* (0.1mg/ml)	0.956±0.0143
Treated with n-hexane extract of *K. africana* (0.2mg/ml)	0.846± 0.0220
Treated with n-hexane extract of *K. africana* (0.5mg/ml)	0.646± 0.0472
Treated with n-hexane extract of *K. africana* (1mg/ml)	0.573± 0.0257
Treated with n-hexane extract of *K. africana* (2mg/ml)	0.543±0.00792

Table 1B: Cell viability values of untreated and treated hepg2 cells

Groups & Doses	Values(±SEM)
Untreated	1.46±1.121
Treated with ethanolic extract of *K. africana* (0.1mg/ml)	0.781 ±0.200
Treated with ethanolic extract of *K. africana* (0.2mg/ml)	0.751± 0.0837
Treated with ethanolic extract of *K. africana* (0.5mg/ml)	0.739 ± 0.0809
Treated with ethanolic extract of *K. africana* (1mg/ml)	0.655 ± 0.121
Treated with ethanolic extract of *K. africana* (2mg/ml)	0.767 ± 0.0289

Table 2A: Cell viability values of untreated and treated hepg2 cells

Groups & Doses	Values(±SEM)
Untreated	0.753±0.0289
Treated with n-hexane extract of *K. africana* (1.47mg/ml)	0.373±0.00959

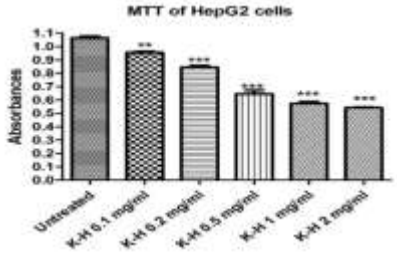
Table 2B: Cell viability values of untreated and treated hepg2 cells

Groups & Doses	Values(±SEM)
Untreated	0.753±0.0289
Treated with ethanolic extract of *K. africana* (1.47mg/ml)	0.327 ±0.00229

Trypan blue assay of n-hexane and ethanolic extracts of *kigelia Africana:* IC50 doses of n-hexane and ethanolic extracts of *K. africana* were applied in trypan blue assay which is best assay to count dead cells, where IC50 dose, 1.47mg/ml of n-hexane extract of *K. africana* was applied for 72 hours. It showed increase in dead cell number as shown in figure 3A. In case of ethanolic extract, *K. africana* increased dead cell number after IC50 dose, 0.153mg/ml treatment of extract after 72 hours as shown in figure 3B.

Figure 1A: MTT activity evaluation by n-hexane extract of *K. africana* (K-H) was done by using five different concentrations, 0.1 mg/ml, 0.2 mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml. Apoptosis of HepG2 cells after treating with K-H assessed by MTT assay between untreated and treated HepG2 cells where treated HepG2 cells or Treated with K-H at different amount of doses which is showing different rate of apoptosis. Values taken were expressed as mean **±** SEM.

1A



Muse analysis by kigelia africana treatment: The results of cell count & viability are shown in figure 4 where cells were treated with IC50 values of n-hexane and ethanolic extracts of *K. africana* which showed less number of live cells in treated group as compared with untreated. Ethanolic extract killed more cells as compared to n-hexane and less live cells were observed. In case of untreated group, 80.8% are viable cells and dead cells are 19.2%. In case of n-hexane extract of *K. africana* treated cells 39.2% are live cells and dead cells are 60.8% and in case of ethanolic extract of *K. africana* 35% are live cells and 65% are dead cells were observed.

Figure 1B: C50 value via MTT activity evaluation by n-hexane extract of *K. africana* (K-H), which is 1.47mg/ml. IC50 was evaluated after treating with K-H and assessed by MTT assay between untreated and treated HepG2 cells

1B

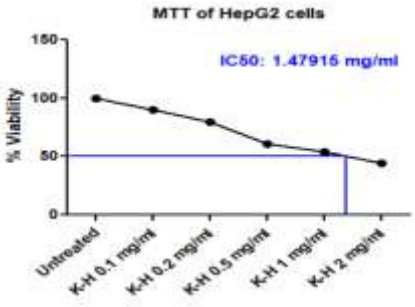


Figure 2A: MTT activity evaluation by ethanolic extract of *K. africana* (K-E) was done by using five different concentrations, 0.1 mg/ml, 0.2 mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml. Apoptosis of HepG2 cells after treating with K-E assessed by MTT assay between untreated and treated HepG2 cells where treated HepG2 cells were treated with K-E at different doses showing apoptosis. Values taken were expressed as mean **±** SEM.

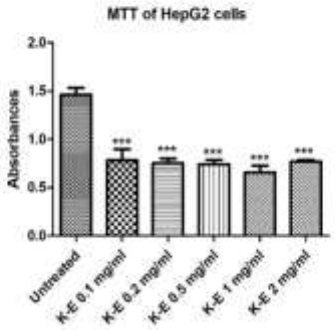


Figure 2B: showing IC50 value of ethanolic extract of *K. africana* (K-E) via MTT assay which is 0.1531 mg/ml. Apoptosis was checked after treating with K-E assessed by MTT assay between untreated and treated HepG2 cells where treated HepG2 cells are treated with K-E at IC50 dose which is showing apoptosis

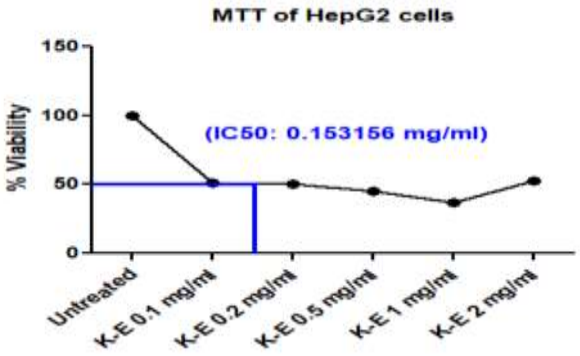


Figure 2A: Crystal violet activity evaluation by n-hexane extract of *K. africana* (K-H) was done by using IC50 concentration, 1.47mg/ml. Apotosis of HepG2 cells after treating with K-H assessed by CV assay between untreated and treated HepG2 cells where treated HepG2 cells are treated with K-H at different amount of doses which is showing different rate of apoptosis. Values taken were expressed as mean ± SEM

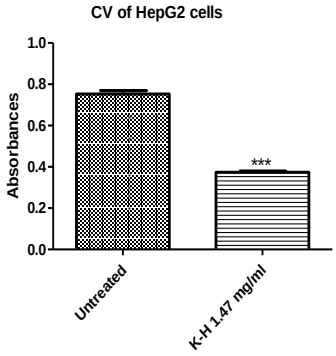


Figure 2B: Crystal violet activity evaluation by ethanolic extract of *K. africana* (K-E) was done by using IC50 concentration, 0.153mg/ml. Apoptosis of HepG2 cells after treating with K-E assessed by CV assay between untreated and treated HepG2 cells where treated HepG2 cells are treated with K-E at IC50 dose which is showing apoptosis. Values taken were expressed as mean ± SEM.

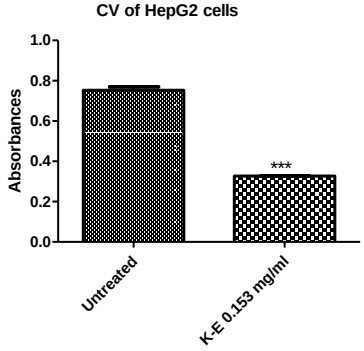


Figure 3A: trypan blue assay evaluation by n-hexane extract of *K. africana* (K-H) was done by using IC50 concentration, 1.47mg/ml. Death rate of HepG2 cells after treating with K-H assessed by trypan blue assay between untreated and treated HepG2 cells where treated HepG2 cells were treated with K-H at IC50 dose which is showing more death rate as compared to untreated group.

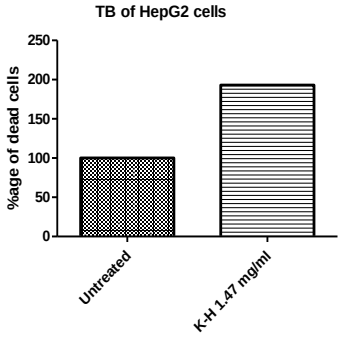


Figure 3B: Trypan blue assay evaluation by ethanolic extract of *K. africana* (K-E) was done by using IC50 concentration, 0.153mg/ml. Death rate of HepG2 cells after treating with K-E assessed by trypan blue assay between untreated and treated HepG2 cells where treated HepG2 cells were treated with K-E at IC50 dose which is showing more death rate as compared to untreated group.

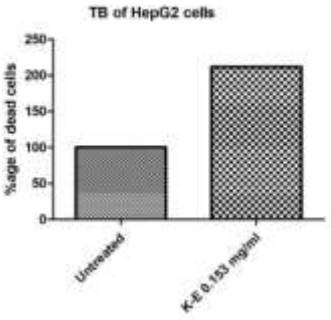
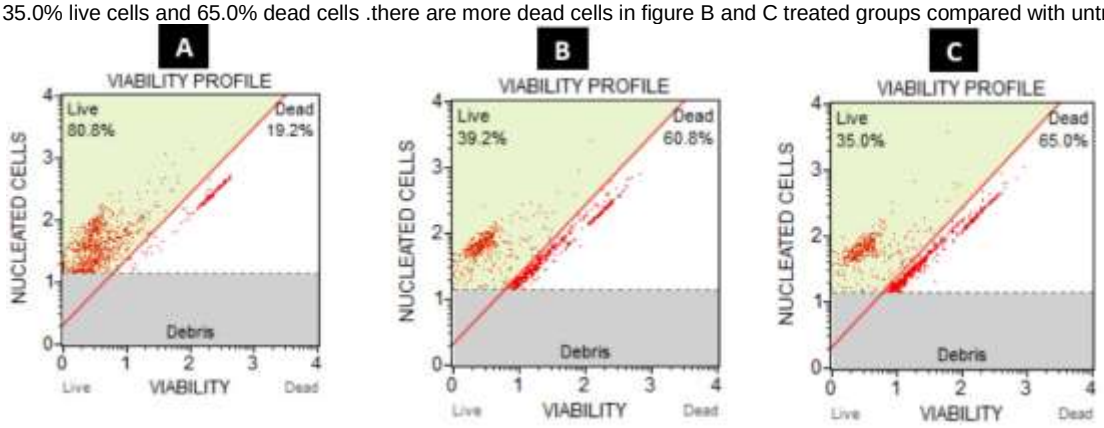


Figure 4: Cell viability where A is untreated showing 80.08% live cells and 19.02% are dead cells in case of, B treated with n-hexane extract of *K. africana* showing 39.02% live cells and 60.08% dead cells and figure C treated with ethanolic extract of *K. africana* which is showing 35.0% live cells and 65.0% dead cells .there are more dead cells in figure B and C treated groups compared with untreated group A



DISCUSSION

Cancer is a condition in which there is failure of apoptosis in tumorous cells due to certain factors. Every cell in living body has its own task which it performs according to microenvironment in body. Similarly, cells grow and multiply in appropriate manner. If due to some factors, cells get some injury, this situation is compensated with production of new young cells. Cancerous condition is initiated when there is uncontrolled proliferation of cells after rapid multiplication. This is also major reason for every type of cancer cell production. Growth of cancerous cell is also different as compared to normal cells. Cancer cells divide continuously. They contain unique characteristics which normal cells do not contain such as leaving of localized area and penetration into other tissues[17-18].

Sometimes, cancer is regarded as tumor, depends on nature, which may be benign or malignant. Usually benign tumors are slow in growth and sometimes show penetration. Hence, these tumors are not considered as fatal and can easily be removed via surgery. But in certain cases, benign tumor can be dangerous if there is less space for its expansion, for example benign tumors present in skull or colon can be much more risky[19]. Certain microbes were found to be causative agents in some cancers such as virus and bacteria. Examples include human T-lymphotropic virus, Epstein-Barr virus, hepatitis B virus, hepatitis C virus, human papilloma virus and *Helicobacter pylori*[20-21]. Hypoxia and scarcity in nutrition are the two additional key factors in cells which can contribute to malignancy as mitochondrial ROS production enhanced due to these factors[22].

Over last decade, it has been confirmed that incidence of hepatocellular carcinoma (HCC) is intensifying in various regions of the world and is sixth most common malignancy. HCC is regarded as major contributor to death due to cancer. Several treatments for HCC are available such as systemic targeted agents, radio embolization, transarterial chemoembolization, radiofrequencey ablation, surgury and transplantation. But certainly, these treatments have some limitations and side effects. So, there is dire need of alternate therapies such as natural medicine, prepared from medicinal plants. Medicinal and aromatic plants have ancient history of therapuetic purposes and many drugs have been prepared to prove valuable for drug expansion program. For many years, medicinal plants had been in focus to discover effective novel anticancer constituents[23]. There is rise in use of naturally occuring anticancer agents in different types of cancers all over the world. Several plant based extracts have been reported to contain anticancer properties and cancer reducing effects.

The previous studies described that plant extracts are anti-proliferative, anti-cancerous, and anti-angiogenic. This was the basis of current study in which anti-cancerous ability of extracts of leaves of *K. Africana* was evaluated. Medicinal plants have much therapeutic value at vast scale in conventional medicine. Many available drugs have been prepared from these medicinal plants[24]. *K. africana* have much traditional uses in various ailments. Therefore, the present study was investigated by using leaf n-hexane and ethanolic extracts of *K. Africana* to evaluate its anticancer properties against liver cancer cell line.

The study observed anticancer potential of *K. africana* on HepG2 human liver cancer cells growth using MTT assay. As shown in current study, *K. Africana* reduced cell viability of HepG2 cells. MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a cytotoxic assay in which conversion of MTT into formazan crystals takes place which depends upon viability of cells that whether their mitochondria is in functional state or not. In this way, condition of cells is determined after treatment.

Cell viability assay, with crystal violet and trypan blue were also done where *K. Africana* extracts of n-hexane showed less live cells in case of crystal violet and more dead cells in case of trypan blue. Furthermore analysis was performed which also showed *K. africana* plant extracts with positive results as compared with untreated ones.

REFERENCES

1. Kakar MI, Collins AS, Mahmood K, Foden JD, Khan M. U-Pb zircon crystallization age of the Muslim Bagh ophiolite: Enigmatic remains of an extensive pre-Himalayan arc. Geology. 2012 Dec 1;40(12):1099-102.
2. Davidson-Hunt I. Ecological ethnobotany: stumbling toward new practices and paradigms. MASA J. 2000;16(1):1-3.
3. Saini SK, Saini RP. Development of correlations for Nusselt number and friction factor for solar air heater with roughened duct having arc-shaped wire as artificial roughness. Solar Energy. 2008 Dec 1;82(12):1118-30.
4. Grace G. Catholic schools: Mission, markets, and morality. Routledge; 2002 Nov 1.
5. El–Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007 Jun 1;132(7):2557-76.
6. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. Hepatology. 2003 Feb;37(2):429-42.
7. Bruix J, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. Hepatology. 2002 Mar;35(3):519-24.
8. Miller AB, Hoogstraten BF, Staquet MF, Winkler A. Reporting results of cancer treatment. cancer. 1981 Jan 1;47(1):207-14.
9. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. Journal of the National Cancer Institute. 2000 Feb 2;92(3):205-16.
10. Lau WY, Leung TW, Liew CT, Ho SK, Simon CH, Tang AM. Preoperative systemic chemoimmunotherapy and sequential resection for unresectable hepatocellular carcinoma. Annals of surgery. 2001 Feb;233(2):236.
11. Gomez-Lechon MJ, Donato MT, Lahoz A, Castell JV. Cell lines: a tool for in vitro drug metabolism studies. Current drug metabolism. 2008 Jan 1;9(1):1-1.
12. Teresa Donato M, Jover R, Jose Gomez-Lechon M. Hepatic cell lines for drug hepatotoxicity testing: limitations and strategies to upgrade their metabolic competence by gene engineering. Current drug metabolism. 2013 Nov 1;14(9):946-68.
13. Aden DP, Fogel A, Plotkin S, Damjanov I, Knowles BB. Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. Nature. 1979 Dec;282(5739):615-6.
14. Knowles BB, Howe CC, Aden DP. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. Science. 1980 Jul 25;209(4455):497-9.
15. Maqbool T, Awan SJ, Malik S, Hadi F, Shehzadi S, Tariq K. In-vitro anti-proliferative, apoptotic and antioxidative activities of medicinal herb Kalonji (Nigella sativa). Current Pharmaceutical Biotechnology. 2019 Dec 1;20(15):1288-308.
16. Hadi F, Awan SJ, Tayyeb A, Maqbool T, Shehzadi S, Malik S, Kausar H, Malik A. Hepato-protective role of itraconazole mediated cytochrome p450 pathway inhibition in liver fibrosis. Pakistan Journal of Pharmaceutical Sciences. 2020 Nov 2;33.
17. Meier P, Finch A, Evan G. Apoptosis in development. Nature. 2000 Oct;407(6805):796-801.
18. Steller H. Mechanisms and genes of cellular suicide. Science. 1995 Mar 10;267(5203):1445-9.
19. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: a cancer journal for clinicians. 2014 Jan;64(1):9-29.
20. Peter S, Beglinger C. Helicobacter pylori and gastric cancer: the causal relationship. Digestion. 2007;75(1):25-35.
21. Weber JL, Wong C. Mutation of human short tandem repeats. Human molecular genetics. 1993 Aug 1;2(8):1123-8.
22. Ralph SJ, Rodríguez-Enríquez S, Neuzil J, Saavedra E, Moreno-Sánchez R. The causes of cancer revisited:"mitochondrial malignancy" and ROS-induced oncogenic transformation–why mitochondria are targets for cancer therapy. Molecular aspects of medicine. 2010 Apr 1;31(2):145-70.
23. Alabi MA, Muthusamy A, Kabekkodu SP, Adebawo OO, Satyamoorthy K. Anticancer properties of recipes derived from Nigeria and African medicinal plants on breast cancer cells in vitro. Scientific African. 2020 Jul 1;8:e00446.
24. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. Journal of advanced pharmaceutical technology & research. 2011 Apr;2(2):104