African Pharmaceutical Journal

journal homepage: journals.uofg.edu.sd/index.php/apj

Original Article

Phytochemical Constituents and *In-Vitro* Anticancer Activity of *Acacia Nubica* Benth. Seeds Ethanol Extract Against Human Breast Cancer Cell Lines

Salma Hago[1], Tang Lu[2], Abdelgadir A. Abdelgadir[1,4], Sitelbanat Yassin[3], Elhadi M. Ahmed[4], Hanmei Xu[2]*

[1]*Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Gezira State, Sudan.*

[2]*The Engineering Research Center of Peptide Drug Discovery and Development, China Pharmaceutical University, Nanjing, Jiangsu 210009, P.R. China.*

[3]*Department of Pharmaceutics, Faculty of Pharmacy, University of Gezira, Gezira State, Sudan.*

[4]*Medicinal and Aromatic Plant Research Center, Faculty of Pharmacy, Gezira University, Gezira State, Sudan.*

Abstract

Breast cancer (BC) is a neoplasm that occurs most frequently among women. It is regarded as a local and international health risk, particularly in developing nations like Sudan. There is global interest in finding novel, naturally occurring anticancer drugs with minimal side effects. This study has been designed to study phytochemical composition and explore the potential cytotoxic effects of *Acacia nubica* Benth. Seeds extract against two breast cancer cell lines and one normal cell line. Seeds extract was prepared using the ultrasonic-assisted extraction method, and the phytochemical constituents were screened using GC-MS and HPLC. The ethanol extract was screened for in vitro antiproliferative properties by using the MTT assay against human breast estrogen receptor-positive MCF-7, triple-negative MDA-MB-435 cancer cell lines, and human umbilical vein endothelial cells (HUVEC) normal cell lines. Oleoyl chloride, oleic acid, linoleic acid palmitic acid, and myristic acid were the major components detected by GC-MS analysis while gallic acid is the only phenolic compound identified by HPLC. The ethanol extract exerted a selective great effect against MDA-MB-435 with IC_{50} equal to 0.050±0.003 with high selectivity index (SI) value > 1000. Overall, these results need to be supported by further studies including *in silico* and *in vivo* studies to evaluate the possible anti-cancer properties of *Acacia nubica* seeds extract and its constituents.

Keywords

Acacia nubica, GC-MS analysis, Breast cancer, MTT assay, MCF-7, MDA-MB-435 Cell lines.

1 Introduction

Cancer is a global health concern, affecting million people around the world, by 2035 this count may reach 24 million [1]. According to the GLOBOCAN 2020 record, there were 2.3 million newly diagnosed breast cancer cases [2]. BC cases have slightly increased in Sudan. BC is the most prevalent cancer among Sudanese women, accounting for 34% of cancer cases in 2017, according to the Khartoum State Disease Registry [3]. Triple-negative breast cancer (TNBC) accounts for 17% of diagnosed BC cases. TNBC is associated with a diverse group of aggressive malignancies that were linked with a decreased prognosis. TNBC is defined by the absence of the estrogen receptor, progesterone receptor, and HER2 overexpression [4].

The advancement in diagnostic and chemotherapeutic agent treatment has increased the survival time of patients with estrogen-dependent breast cancers. As metastatic cancerous cells defy chemotherapy, estrogen-independent breast cancer has a poor prognosis. New cancer research treatment approaches should be pursued [5]. Sudan is home to a wide range of medicinal plants and a diversified ecosystem [6].

Aromatic plants are receiving great attention due to their biological and chemical diversity. They are used traditionally for the treatment of inflammation, microbial infections, and other various diseases [7].

The species *Acacia nubica* (A. nubica) is a member of the Fabaceae family. It is a shrub with basal branches that is green-gray or whitish and spinescent. It is found in

Received: 30 March 2023; Revised: 20 June 2023; Accepted: 24 June 2023

Corresponding author
* Salma Ahmed Hago. E-mail: Hago.selma@yahoo.com

Northern and Central Sudan under the name La' ot. The fruits (pods and seeds) of *A. nubica* contain polyphenolic constituents like methyl gallate, gallic acid, and digallic acid [8].

The genus *Acacia* belongs to the subfamily Mimosoideae and encompasses significant trees and shrubs.

Seed extracts of different *Acacia* species exert significant antitumor effects against different cancer cells. *A. hydaspica* seeds extract contained different phenolic compounds and exhibited significant activity against PC-3 prostate and MDA-MB-231 breast cancer cell lines [9]. Gallocatechin 5-O-gallate was responsible for the anti-uveal melanoma effects of the methanolic extract of *A. nilotica*, which showed antiproliferative activity against uveal melanoma [8].

The purpose of this study was to use the GC-MS and HPLC techniques to identify the chemical components and to assess the in vitro cytotoxic effects of *A. nubica* seeds extract on MCF-7, MDAMB-435 metastatic mammary adenocarcinomas, and HUVEC human normal cells.

2 Materials and methods

2.1. Plant material

Seeds of *A. nubica* were harvested from trees in Gezira state, Sudan. In the herbarium of the Medicinal and Aromatic Plant Research Center, Faculty of Pharmacy, University of Gezira, Sudan, a representative sample of the plant material was taxonomically recognized and preserved.

2.2. Chemicals and reagents

MTT, DMEM, FBS, penicillin/streptomycin, and trypsin-EDTA were procured from Gibco, USA, as well as other materials and chemicals for the MTT assay. Standard reference materials for HPLC analysis were purchased from Sigma-Aldrich in Germany including gallic acid, catechin, chlorogenic acid, rutin, ellagic acid, hesperidin, quercetin, kampeferol, and apigenin. Other substances were of analytical quality.

2.3. Plant extraction

Coarsely powdered dried plant seeds (100 g) was extracted using an ultrasonic-assisted extraction apparatus and ethanol 95%. The extract was subsequently filtered and evaporated using rotatory evaporator equipment under reduced pressure.

2.4. Gas chromatography- mass spectrometry (GC-MS) analysis

The seeds extract was assessed using a GC-MS instrument (Model GC-MS-QP22010-Ultra, Shimadzu Company, Japan) that used a silica capillary stationary phase with an internal diameter of 0.25 mm, a film thickness of 0.25 μm for the analysis and 30m length. The temperature of the GC oven was first set at 60 °C and increased steadily at a rate of 10 °C/min to 300 °C. The sample was injected in split mode at a flow rate of 50 ml/min using helium as the carrier gas. The ion source was maintained at 200°C, while the mass selective detector transfer line heater was retained at 250°C. By comparing retention time and mass spectrum data, the GC-NIST MS's mass spectral library was used to identify the various compounds.

2.5. HPLC analysis of flavonoids and phenolic acids

Using Waters 2690 Alliance HPLC system with a Waters 996 photodiode array detector and an analytical column C18 (4.6x250mm, 5μm) at room temperature, the analysis of flavonoids and phenolic acids compounds was completed. Using a mobile phase of 0.1% phosphoric acid in water: acetonitrile after gradient elution at a flow rate of 1 ml/min and wavelength set at 280 nm for 80 minutes, the separation was carried out. 10 μl of each sample was injected after a stock solution of 9 standards and *A. nubica* extract were separately diluted in methanol and filtered through a 0.22 m syringe filter. By comparing their retention periods to those of a group of external standards that were also evaluated under identical circumstances, flavonoids and phenolic acids were identified.

2.6. Cell lines and cell culture

MCF-7, MDA-MB-435, and HUVEC normal cells were provided by Nawah-Scientific in Cairo, Egypt and China Pharmaceutical University in Nanjing, Jiangsu, respectively. DMEM media mixed with 10% FBS, 100 units/mL of penicillin, and 100 mg/mL of streptomycin was used to sustain the cells after they were quickly removed from liquid nitrogen. All cells were placed in a 37°C, 5% carbon dioxide-containing incubator with a controlled humidity level and temperature.

2.7. Cell cytotoxic assay

For the cell cytotoxicity experiment, a total of 100 μl of fresh culture media containing live cells were placed in a 96-well plate. The seeds extract had been dissolved in DMSO to create a stock solution and serial dilutions, 100 μl of various concentrations (1–1000 g/ml) were added to the wells, and cells were then grown for an additional 72 hours. Then, for the next 4 hours at 37°C, each well was incubated with 20 μl of MTT reagent (5 mg/ml) in phosphate buffer saline (PBS). The medium was then removed, 150 μl of MTT was added, and the cells were shaken for 15 min on an orbital shaker. After removing the medium and removing the formazan produced by metabolic reactions. Using a microplate spectrophotometer (BMGLABTECH®FLUOstar Omega, Germany), the optical density of active cells in DMSO was determined at 570 nm. [10].

2.8. Statistical Analysis

GraphPad Prism v5 was used to determine the IC_{50} findings, which were presented as mean ± standard error of the mean (SEM).

3 Results

3.1 GC-MS analysis of plants extracts

A. nubia's GC-MS chemical profile revealed the presence of 36 phytomolecules (Figure 1); 12 significant peaks were found, including oleoyl chloride (20.39%), diethoxymethyl acetate (12.61%), myristic acid (10.89%), linoleic acid (10.37%), bis(2-ethylhexyl) phthalate (8.17%), and palmitic acid. Beta-monoglyceride (6.93%), oleic acid (5.72%), fumaric acid, 2-dimethylaminoethyl nonyl ester (2.36%), 1-fluorononane (1.76%), ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- (1.28%), and ethyl palmitate (1.19%) (Table 1).

3.2. HPLC analysis of flavonoids and phenolic acids

HPLC screening of *A. nubica* extract declared the occurrence of gallic acid (48.05 %) as the only phenolic compound identified in the ethanolic extract as shown in (Figure 2) compare to the standards chromatogram (Figure 3).

Table 1: GC-MS analysis of the *A. nubica* ethanolic extract

Peak	Compound	Retention Time	Peak Percentage
1	Diethoxymethyl acetate	3.350	12.61
2	Pyridine, 3-methyl-	3.887	0.99
3	Oxirane, (ethoxymethyl)-	4.017	0.31
4	2-Pentanone, 4-hydroxy-	4.080	0.92
5	Ethanol, 2-propoxy-	4.222	0.89
6	3,4-Epoxytetrahydrothiophene-1,1-dioxide	4.430	0.39
7	1,4-Cyclohexanediol, trans-	4.525	0.40
8	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	6.450	0.54
9	2-Cyclohexenol, 4-acetamido-, E\\trans-	7.098	0.50
10	Methyl 2-acetamidoacrylate	7.383	0.90
11	2-Methoxy-4-vinylphenol	10.284	0.40
12	Nonanoic acid, 9-oxo-, ethyl ester	11.543	0.31
13	1-Tetradecene	11.686	0.49
14	1-Dodecanol	12.639	3.63
15	Phenol, 3,5-bis(1,1-dimethylethyl)-	13.095	0.58
16	3-tert-Butyl-4-hydroxyanisole	13.590	3.26
17	2-Deoxy-D-galactose	14.033	0.26
18	.alpha.-D-Glucopyranoside, O-.alpha.-D-gluc	14.080	0.19
19	Dodecanoic acid, 3-hydroxy-	14.230	0.17
20	Hydrazinecarboxamide, N,N-diphenyl-	14.350	0.27
21	1-Fluorononane	15.343	1.76
22	Myristic acid	18.380	10.89
23	Ethyl palmitate	18.656	1.19
24	Propanoic acid, 3-mercapto-, dodecyl ester	18.776	0.54
25	Linoleic acid	20.070	10.37
26	Oleic Acid	20.144	5.72
27	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	20.274	1.28
28	Fumaric acid, 2-dimethylaminoethyl octadec	21.396	0.66
29	Undecanoyl chloride	21.605	0.38
30	Hexadecanoic acid, 1-[[[(2-aminoethoxy)hyd	21.672	0.36
31	Fumaric acid, 2-dimethylaminoethyl nonyl ester	22.843	2.36
32	Palmitic acid .beta.-monoglyceride	23.232	6.93
33	Bis(2-ethylhexyl) phthalate	23.544	8.17
34	Oleoyl chloride	24.690	20.39
35	alpha.-Monostearin	24.855	0.95

3.3. Cell cytotoxic assay

After 72 hours of treatment, the MTT assay was used to determine the cytotoxic effects of *A. nubia* ethanol extract against the BC cell lines MCF-7 and MDA-MB345 as well as a normal cell line (HUVEC). The in vitro growth inhibitory cytotoxic concentrations (IC_{50}) and selectivity index (SI) of plant extract were then calculated. IC_{50} values for A. nubica against MDA-MB-435, MCF-7, and HUVEC cell lines were 0.050±0.003, 37.11±0.18, and 525.9 µg/ml, respectively. Table 2: Strengths of the clinical pharmacy service at NCI.

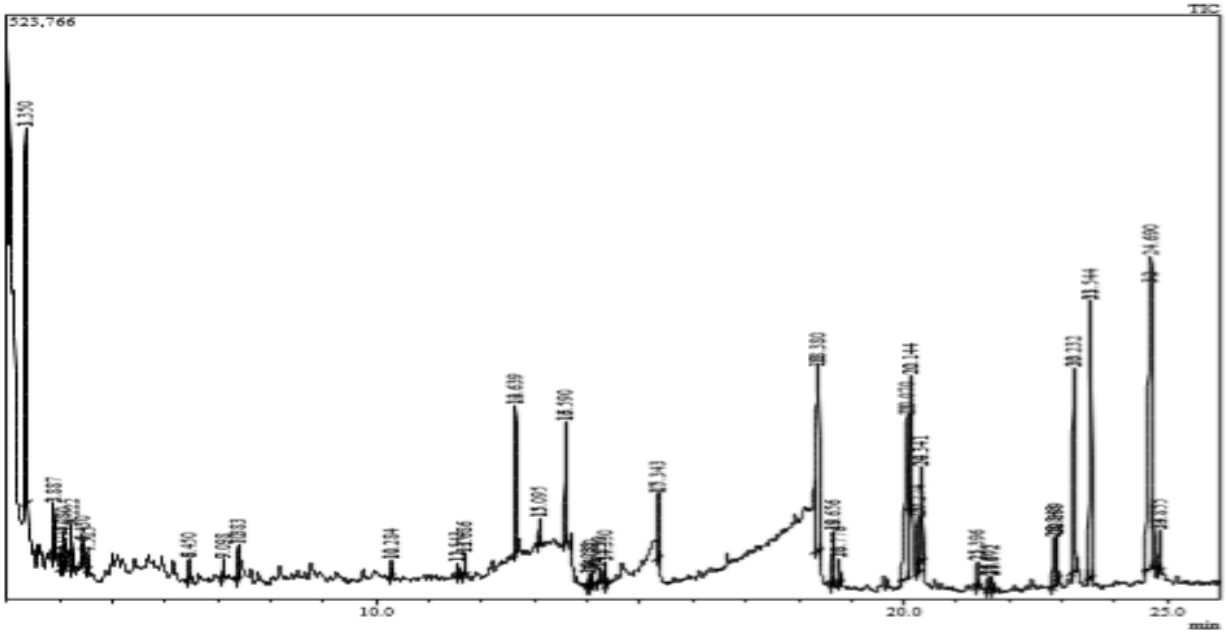

Figure (1): Knowledge of HCPs about the benefits of the presence of the clinical pharmacists (n=68).

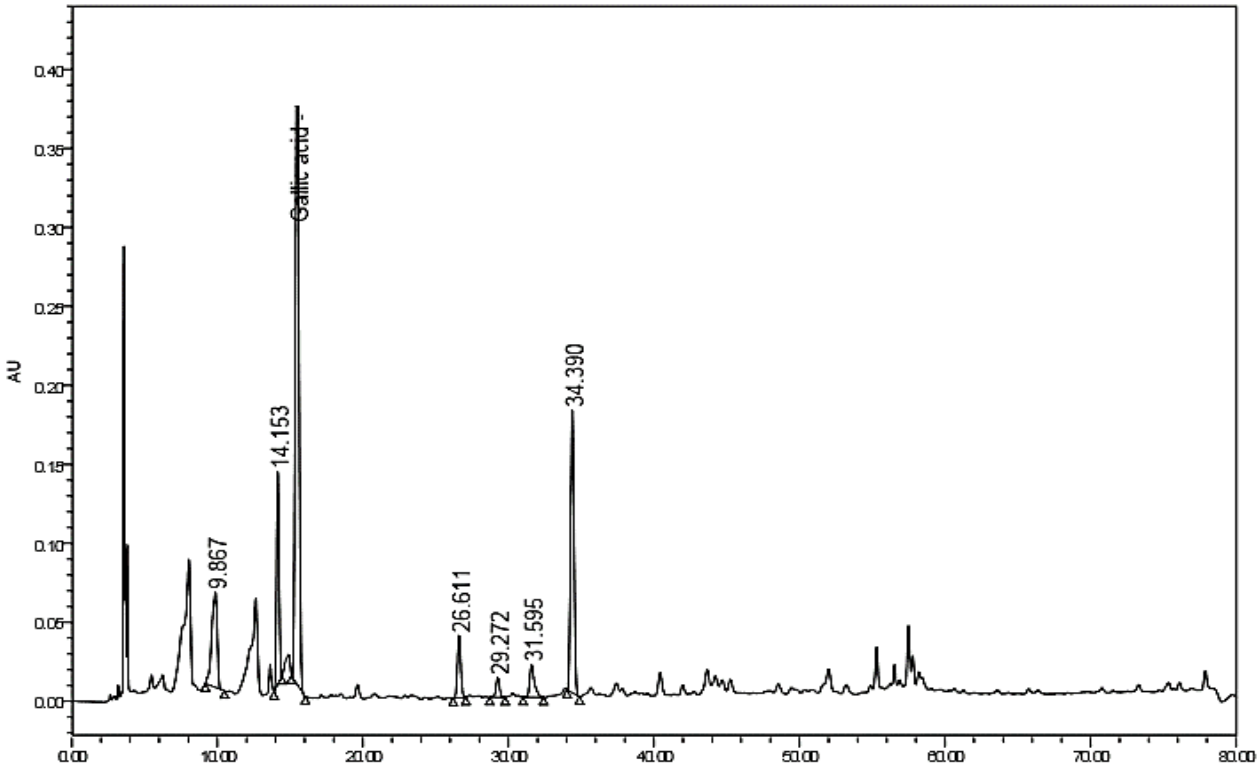

Figure (2): HPLC Chromatogram of *Acacia nubica* ethanolic extract.

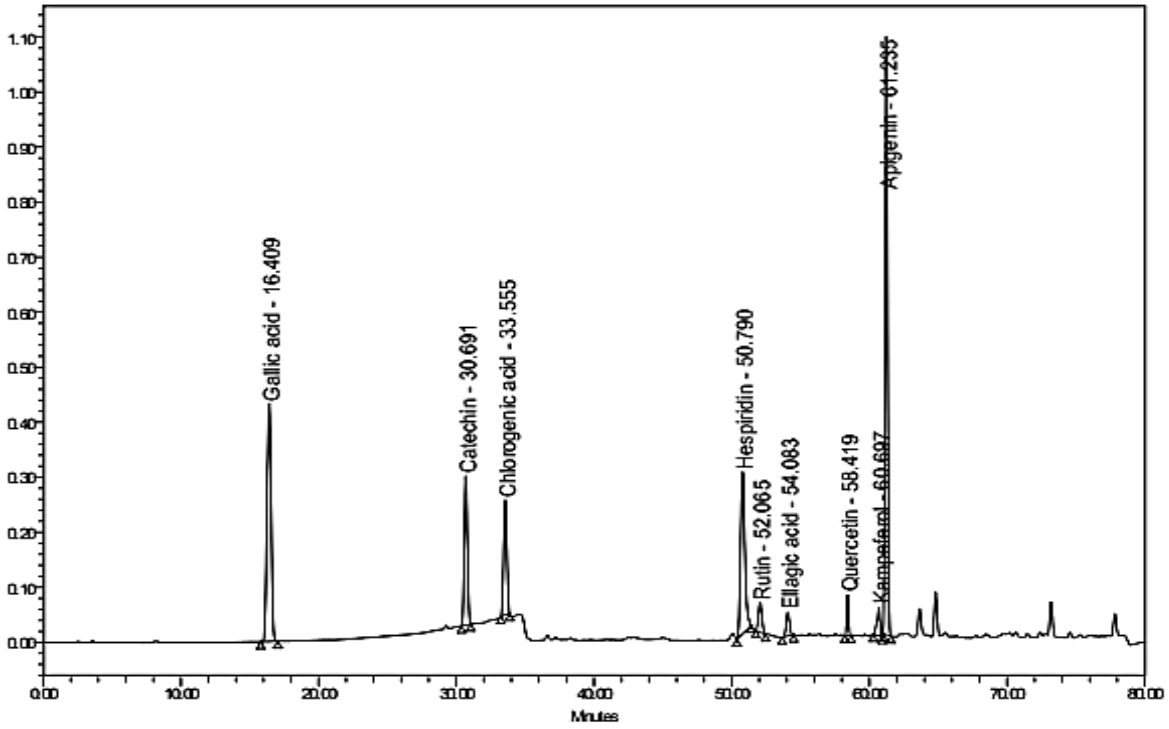

Figure (3): HPLC Chromatogram of phenolic and flavonoids standards.

4 Discussion

There have been a few investigations exploring the anticancer potential of plants used in traditional Sudanese medicine. The chemical composition and *in vitro* anticancer potential of *A. nubica* seeds are described in this research. However, according to our knowledge this is the initial study held on phytochemical analysis and cytotoxic effects of seeds of the *A. nubica*.

The chemical composition of the extract was analyzed by GC-MS and HPLC. GC-MS revealed the existence of oleoyl chloride which belongs to the class of organic compounds known as acyl chlorides, unsaturated fatty acids like oleic and linoleic and saturated fatty acids (palmitic and myristic acid) were found in significant proportion. Similarly, GC-MS conducted by Biswal *et al*. 2020 and Abdel Karim *et al*. 2021 on seeds extracts of *Acacia planifrons* and *Acacia polyacantha* revealed the presence of different fatty acids derivatives like pentadecanoic acid, 14-methyl, methyl ester, 9,12-octadecadienoyl chloride (Z,Z) (46.6%), heptacosanoic acid, methyl ester (4.72%), E-2 octadecadecen-1-ol 9,12-octadecadienoic acid (Z,Z) methyl ether , hexadecanoic acid, methyl ester methyl stearate, and eicosanoid acid, methyl ester [11, 12].

The HPLC assessment of *A. nubica* ethanol extract was performed to identify flavonoids and phenolic acids contents, according to the International Organization for Standardization. Gallic acid is the only phenolic compound detected by HPLC in *A. nubica* seed extract, it was also detected by Salem *et al*., 2011 in fruits (pods and seeds) of the plant [8].

A. nubica seed extract exerted a potent selective cytotoxic effect against metastatic MDA-MB-435 and a moderate cytotoxic effect against MCF-7 cell line at IC50 equal to 37.11±0.18, with selectivity indexes equal >1000 and 14.17, respectively, so the use of extract against BC was considered in safe.

A. hydaspica seeds extract and its components methyl gallate, 7-O-galloylcatechin, and catechin-3-O-gallate obtained gave a positive impact when they were examined on certain breast cancer cell signaling pathways and cell survival. They could be used for the treatment and prevention of breast cancer [9].

5 Conclusion

The study was conducted to investigate the chemical profile and to evaluate the cytotoxic effects of *A. nubica* seeds ethanolic extract on BC and normal cell lines. The seeds extract contained phenolic acid and different fatty

acids phytomolecules. It displayed selective antiproliferative activity against estrogen receptor-positive MCF-7 and metastatic triple-negative (MDA-MB-435) types of BC cell lines. More *in vivo* research is recommended to investigate the possible anti-carcinogenic benefits of *A. nubica* extract and its components.

References

1. Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A. and Bray, F. (2021a). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*. 71(3): 209–249.

2. Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A. and Bray, F. (2021b). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*. 71(3): 209–249.

3. Saberian, M.; Mehrabani, K. and Shahraki, H. R. (2021). Clustering time trends of breast cancer incidence in Africa: a 27-year longitudinal study in 53 countries. *African Health Sciences*. 21(1): 47–53.

4. Yadav, B. S.; Chanana, P. and Jhamb, S. (2015). Biomarkers in triple negative breast cancer: A review. *World Journal of Clinical Oncology*. 6(6): 252.

5. Iqbal, J.; Abbasi, B. A.; Batool, R.; Mahmood, T.; Ali, B.; Khalil, A. T.; Kanwal, S.; Shah, S. A. and Ahmad, R. (2018). Potential phytocompounds for developing breast cancer therapeutics: Nature's healing touch. *European Journal of Pharmacology*. 827:125–148.

6. Khalid, H.; Abdalla, W. E.; Abdelgadir, H.; Opatz, T. and Efferth, T. (2012). Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. *Natural Products and Bioprospecting 2012 2:3*. **2**(3): 92–103.

7. El-Ghazali, G.E., El-Tohami, M.S. and El-Egami, A. A. (1994). *Medicinal plants of the Sudan: medicinal plants of the White Nile provinces.* National Centre for Research, Medicinal & Aromatic Plants Research Institute.

8. Salem, M.M; Davidorf, F.H.; Abdel-Rahman, H.M. (2011). *In vitro* anti-uveal melanoma activity of phenolic compounds from the Egyptian medicinal plant *Acacia nilotica*. Fitoterapia. 82(8):1279-1284.

9. Afsar, T.; Trembley, J. H.; Salomon, C. E.; Razak, S.; Khan, M. R. and Ahmed, K. (2016). Growth inhibition and apoptosis in cancer cells induced by polyphenolic compounds of *Acacia hydaspica*: Involvement of multiple signal transduction pathways. *Scientific Reports 2016 6:1*. 6(1): 1–12.

10. van de Loosdrecht, A. A.; Beelen, R. H. J.; Ossenkoppele, G. J.; Broekhoven, M. G. and Langenhuijsen, M. M. A. C. (1994). A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. *Journal of Immunological Methods*. **174**(1–2): 311–320.

11. Abhishek Biswal, R.; Fernando, L.; Pazhamalai, V. and Brindha Devi, P. (2020). Phytochemical screening and GC-MS analysis of ethanolic extract of *Acacia planifrons* seeds. *Research Journal of Pharmacy and Technology*. **13**(10): 4823–4825.

12. Abdel Karim, M., Hamid, A. M., & Hagr, T. E. (2021). Gas Chromatography/Mass Spectroscopy Analysis, antibacterial Activity of Fixed Oil from *Acacia polyacantha* (Sudanese Kakamout) Seeds. *Sudan Online Research Journal*, 21-7.