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Original Research Article

Phytochemical Constituents and *In-Vitro* Anticancer Activity of some Medicinal Plant Extracts against MCF-7 and MDA-MB-435 Human Breast Cancer Cell Lines

Salma Hago[1]*, Tang Lu[2], Abdelgadir A. Abdelgadir[1], 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*

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ABSTRACT

Breast cancer (BC) is a malignancy, considered as a global and local health hazard. Searching for new naturally occurring anticancer medications with few adverse effects is attracting attention worldwide. This study aimed to screen the phytochemical components of ethanol extracts of eight Sudanese medicinal plants (*Ocimum basilicum* leaves, *Mentha longifolia leaves*, *Linum usitatissimum* seeds, *Allium sativum* L bulb, *Nigella sativa* seeds, *Piper nigrum* L. fruits, *Zingiber officinale* rhizome, and *Foeniculum vulgare* seeds), to evaluate and compare their cytotoxic effects against two types of BC cell lines and to estimate their safety toward normal cell lines. The extracts of the chosen plants were obtained using an ultrasonic-aided extraction process, and their components were characterized by GC-MS. The cytotoxic effects of ethanol extracts were tested *in vitro* against human breast estrogen receptor-positive MCF-7, triple negative MDA-MB-435, and umbilical vein endothelial cells (HUVEC) normal cell lines using the MTT assay. The ethanol extracts were found to be rich in varied phytochemical components. *Mentha longifolia* and *Ocimum basilicum* ethanol extracts exerted the greatest activity against MDA-MB-435 with IC_{50} equal to 0.7437±0.31 and 16.78±2.47µg/ml, respectively. While *Linum usitatissimum*, *Allium sativum*, and *Nigella sativa* ethanol extracts exhibited significant cytotoxic effects on estrogen receptor-positive MCF-7 type of BC cell line with comparable IC_{50} values of 2.008±0.18, 8.661±0.25 and 10.072±0.18µg/ml, respectively. All ethanol extracts showed weak cytotoxic effects (IC_{50} > 100µg/ml) against HUVEC with high selectivity index (SI) values. These findings potentiate the need for further *in vivo* anti-cancer assessments for the active extracts and their components.

***Keywords*:** Medicinal plants, GC-MS, Breast cancer, MTT assay, MCF-7, and MDA-MB-435 Cell lines .

Introduction

Breast cancer (BC) is a major public health issue worldwide. Following, the GLOBOCAN 2020 predictions, 2.26 million cases were documented in 2020, with an anticipated 2.3 million new cases whereas, female BC has surpassed lung cancer as the most commonly diagnosed disease in women. According to the International Agency for Research on Cancer, BC is the top cause of cancer death in women because it was responsible for malignancies in females worldwide.[1] Triple-negative breast cancer (TNBC) accounts for 17% of the diagnosed breast cancer cases. TNBC is associated with a diverse group of aggressive malignancies that were linked with a decreased prognosis.[2] TNBC is defined by the absence of the estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) overexpression.[3]

*Corresponding author. E mail: xuhanmei6688@126.com
Hago.selma@yahoo.com; salmahago124@gmail.com
Tel: 0024911928119; 0013913925346

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Despite its reputation as a disease mostly affecting industrialized countries, less developed parts of the world account for more than half of breast cancer diagnoses and two-thirds of breast cancer-related fatalities. In most African nations it is on the rise. Women in Sub-Saharan Africa have a substantially higher risk of breast cancer mortality than women in Western nations. Sudan has shown a slight increase in BC cases. According to the Khartoum State Disease Registry, BC is the most frequent cancer among Sudanese women, accounting for 34% of cancer cases in 2017.[4] The situation for women with BC in Central Sudan is worse than in affluent nations, but comparable to African countries.[5] The survival time of women with estrogen-dependent breast tumors can be elevated by the development in diagnosis and treatment by chemotherapeutic agents. While estrogen-independent breast cancer has a poor prognosis because metastatic cancer cells resist chemotherapy. Therefore, new cancer therapeutic approaches should be held.[6]

Natural products have an essential role in the development of novel pharmaceuticals and pharmacological molecules. An ethnomedicinal approach is a valuable tool for finding physiologically active plant-based natural products. [6]

Sudan is home to a diverse ecosystem and a huge variety of medicinal plants. Traditional medicines have a long history in Sudan.[7]

Mentha longifolia L. (*M. longifolia*) family Lamiaceae is a Sudanese medicinal aromatic perennial rhizomatous herb is known as wild mint. [8] Mint extracts possess numerous biological activities, including anticancer, antioxidant, and antifungal activities against *Candida albicans*. [9-10] The volatile oils obtained from *Mentha* species possess cytotoxic activities against the MCF-7 breast cancer cell line.[11] The

biological activity of *Mentha* species has been attributed to the presence of volatile oil constituents, terpene, and phenolic compounds.[12,13]

Ocimum basilicum Linn, (*O. basilicum*) is an annual herb growing in tropical regions all over the world. In Sudan, it is called Rehan and is rich in essential oils with a camphor smell,[14] phenolic compounds, and a wide array of natural products.[15] It is commonly used for the treatment of many diseases, such as headaches, respiratory infections, and diarrhea.[16,17] The reported biological effects of *O. basilicum* essential oils include antioxidant, antiviral, anti-acetyl cholinesterase, anticonvulsant, neuro-protective, and cardio-protective effects.[18-20]

Flax seed *Linum usitatissimum* (*L. usitatissimum*) is a member of genus Linum in the Linaceae family. Flax seeds are abundant in dietary fiber as well as lignans and isoflavones, as well as a variety of minerals and omega-3 fatty acids. [21] Flax oil is advised as part of a well-balanced human diet to help in diseases such as heart disease. [22] The isoflavone and lignin found in extract obtained from *L. usitatissimum* had a favorable effect on the therapy of colon tumors. [23]

Garlic, *Allium sativum* L. (*A. sativum*) is a perennial bulb plant of the Allium genus and family (Amaryllidaceae). Steroids, terpenoids, alkaloids, phenolic chemicals, and carbohydrates were discovered in garlic extracts during phytochemical screening.[24] Its biological activities have mostly been linked to two types of chemicals: polar sulfur compounds and saponins. Various Allium extracts, whether aqueous or alcoholic, were tested as a single agent treatment or in combination with cancer therapeutic drugs in several cancer lines, including, colon cancer, [25] breast and liver cancer, and leukemia. [26]

Black seed or black cumin *Nigella sativa* (*N. sativa*) a medicinal herb is belonging to the Ranunculaceae family.[7] A large number of bioactive compounds, including thymoquinone, alkaloids (nigellidine), phenol (carvacrol), fatty acid (linoleic and palmitic acids), and saponin (alpha-hederin) have been detected in seeds.[27,28] The biological investigations of the seed extracts reveal a broad spectrum of activities including antidiabetic, [29] anti-hypertensive, [30] and anti-inflammatory.[31] The aqueous extract of *N. sativa* is active against the MCF-7 breast tumor cell line. [32]

Black pepper berries *Piper nigrum* L. (*P. nigrum*) are part of the Piperaceae family. The fruits are dried and consumed as a spice. Piperine is the major ingredient and is responsible for pepper's medicinal benefits including anticancer activities.[33] B-caryophyllene, 3-carene, D-limonene, and a-pinene were the volatile oils found in black pepper. [34] The black pepper extracts exhibit antioxidant properties and anti-carcinogenic effects.[35,36]

Zingiber officinale (*Z. officinale*) is a ginger rhizome that belongs to the Zingiberaceae family. The oil yield from the ginger contains sesquiterpene hydrocarbons, alcohols, monoterpene hydrocarbons, and esters. Zingiberene and sesquiphellandrene were the most prevalent detected compounds. [37] The primary phenolic compounds in ginger root are gingerols. They exert anticarcinogenic, antioxidant, and anti-inflammatory effects.[38]

Fennel seed or Shamar *Foeniculum vulgare (F. vulgare*) family Apiaceae is widely used for flavouring foods and beverages because of its pleasant spicy scent. The primary components of *F.vulgare* oil were trans-anethole, according to a gas chromatographic-mass spectrometric study.[39] Nine components were found in ethanol and methanol seed extracts, including linoleic acid, palmitic acid and oleic acid.[40] The oil exhibited moderate antioxidant activities. The essential oil of *F. vulgare* in particular anethole, exhibits cytotoxic activity against human cervical cancer, human colorectal adenocarcinoma, and human breast adenocarcinoma as well as *in vitro* genotoxic and apoptotic activities.[41]

The previously mentioned plants are extensively used in Sudanese traditional medicine because they are affordable medicinally valuable plants. This study was conducted to identify their chemical constituents using the GC-MS technique and to evaluate the *in vitro* cytotoxic effects of their ethanol extracts on two BC cell lines with a different hormone receptor expression, MCF-7 and MDA-MB-435, and on HUVEC human normal cells.

Materials and Methods

Plant materials

The selected fresh/ dried plant samples were obtained in May/2020 from the local market and a private garden in Gezira state, Sudan. The voucher specimens of plant materials *Ocimum basilicum* leaves, *Mentha longifolia leaves*, *Linum usitatissimum* seeds, *Allium sativum* L bulb, *Nigella sativa* seeds, *Piper nigrum* L. fruits, *Zingiber officinale* rhizome, and *Foeniculum vulgare* seeds were taxonomically recognized and kept at the herbarium of Medicinal and Aromatic Plant Research Center, Faculty of Pharmacy, Gezira University, Sudan, under voucher numbers (1552020-1, 1552020-2, 1552020-3, 1552020-4, 1552020-5, 1552020-6, 1552020-7, 1552020-8), respectively.

Chemicals and reagents

The Colorimetric cytotoxic assay reagents and chemicals for MTT (4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin and trypsin-EDTA (ethylenediamine tetraacetic acid), were supplied from Gibco, USA. Other analytical grade chemicals were purchased from Sigma-Aldrich, Germany.

Plant extraction

Fresh/ shade dried plant materials (100 g) were coarsely powdered and extracted by ultrasonic-assisted extraction apparatus using one liter of ethanol 95%. The extracts were then filtered and evaporated under reduced pressure and temperature set at 60ºC using rotatory evaporator apparatus.

Gas chromatography- mass spectrometry (GC-MS) analysis

The ethanol extracts were analyzed with a GC-MS apparatus (Model GC-MS-QP22010-Ultra, Shimadzu company, Japan) with a |(Rtx-5MS) silica capillary stationary phase with a film thickness of 0.25 mm, a length of 30 m and an internal diameter of 0.25 µm. The GC oven's initial temperature was 60°C, which was gradually escalated to 300°C at a rate of 10°C/min. Helium was used as the carrier gas and the samples were injected in split mode at a flow rate of 50 ml/min. The GC's mass selective detector transfer line heater was kept at 250°C, while the ion source was kept at 200°C.[42]

The NIST (National Institute of Standards and Technology) mass spectral library of the GC-MS was used to identify substances by comparing retention time and mass spectral data.

Cell lines and cell culture

MCF-7, MDA-MB-435 cell lines, and HUVEC normal cells were supplied from Nawah-Scientific in Cairo, Egypt, and China Pharmaceutical University in Nanjing Jiangsu province, China, respectively. The cells were rapidly taken from liquid nitrogen and maintained in a DMEM medium supplemented with 10% FBS, 100 unit/mL penicillin, and 100 mg/mL streptomycin. All cells were placed in an incubator with a regulated environment and humidity, which was maintained at 37°C and contained 5% carbon dioxide.

Cell cytotoxic assay

A total of 100 µl of fresh culture media of viable cells were planted in a 96-well plate for the cell cytotoxicity experiment. Following a 24-hour incubation period, 100 µl of different concentrations (1-10000 µg/ml) were applied to the wells after dissolving each plant extract in DMSO (Dimethyl sulfoxide) to obtain a stock solution and make serial dilutions, then cells were cultured for another 72 hours. After that, each well was filled with 20 µl of MTT reagent (5 mg/ml) in phosphate buffer saline (PBS) and incubated at 37°C for 4 hours. Then the media was withdrawn followed by the addition of 150 µl MTT and shaking of the cells on an orbital shaker for 15 min. After withdrawing of media and dissolving the formazan formed by metabolically active cells in DMSO, the optical density was measured at 570 nm using a microplate spectrophotometer (BMGLABTECH®FLUOstar Omega, Germany). [43]

Statistical analysis

The IC_{50} results were calculated using GraphPad Prism v5 and demonstrated as mean ± standard error of the mean (SEM).

Results and Discussion

The chemical analysis of plants ethanol extract was carried out using GC-MS as best approach for identifying bioactive compounds. [44]
The GC-MS analysis of *M. longifolia* leaves ethanol extract. revealed the presence of 13 compounds. Phytol was the most prominent compound found in the highest concentration (54.82%) followed by (-)-carvone (8.54%), α-linolenic acid (7.57%), palmitic acid (3.19%), limonene-6-ol (1.72%) and D-carvone (1.11%) as shown in (Table 1 and Figure 1).
In *O. basilicum* leaves ethanol extract, 17 compounds had been identified by GC-MS (Table 2, Figure 2). Methyl eugenol (40.58%) was a major identified compound followed by tau-cadinol (10.48 %), phytol (6.03%), squalene (6%), and α-tocopherol (2.89%).
The chemical profile of *L. usitatissimum* seed ethanol extract (Table 3, Figure 3) showed the presence of 35 compounds with varied peaks percentage. Where 7-tetradecenal,(Z) (27.47 %) was a major identified compound followed by galactopyranoside, methyl, .alpha.-D- (21.92 %), monolinolenin (11.30 %), palmitic acid (8.33 %) and linolenic acid, ethyl ester (5.28 %). Stigmasterol a phytosterol was found in a lower percentage (0.65%).
The analysis of *A. sativum* bulb ethanol extract revealed the presence of 25 compounds (Table 4, Figure 4). Di-n-octyl phthalate was the most abundant compound (27.53 %). Phytosterol compounds (gamma.-sitosterol, stigmasta-5,24(28)-dien-3-ol, (3.beta.)- and ergost-5-en-3-ol, (3.beta.)-) were found in significant proportion.

Table 1: Major compounds identified by GC-MS analysis in *Mentha longifolia* ethanol extract

Compound	Retention Time	Peak Percentage (%)
D-Carvone	9.260	1.11
(-)-Carvone	9.784	8.54
Palmitic acid	19.188	3.19
Phytol	20.931	20.40
Phytol	21.553	34.42
Linolenic acid	21.714	7.57
Limonen-6-ol, pivalate	23.164	1.72

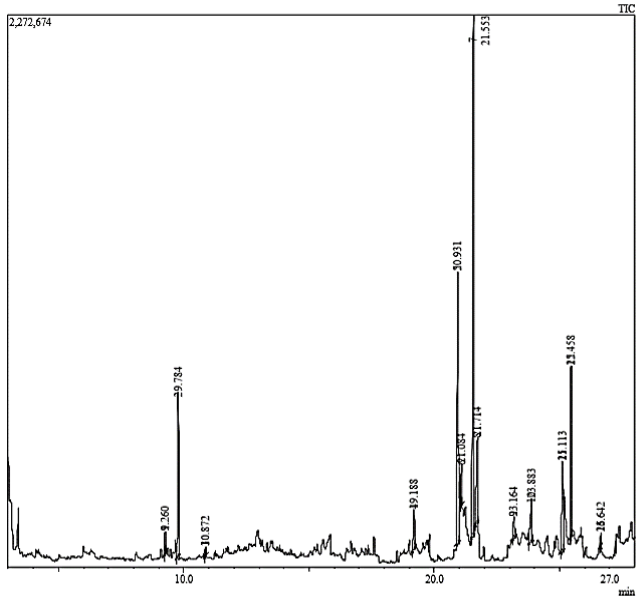

Figure 1: GC-MS chromatogram of the *Mentha longifolia* ethanol extract.

Table 2: Major compounds identified by GC-MS analysis in *Ocimum basilicum* ethanol extract

Compound	Retention Time	Peak Percentage (%)
Methyleugenol	12.244	40.58
tau.-Cadinol	15.952	10.48
Palmitic acid	19.782	2.30
Phytol	21.517	6.03
Linolenic acid	21.685	2.35
Palmitic acid	25.093	1.00
α-Tocopherol	26.165	2.89
Linolenic acid	26.617	1.46
Squalene	27.886	6.00

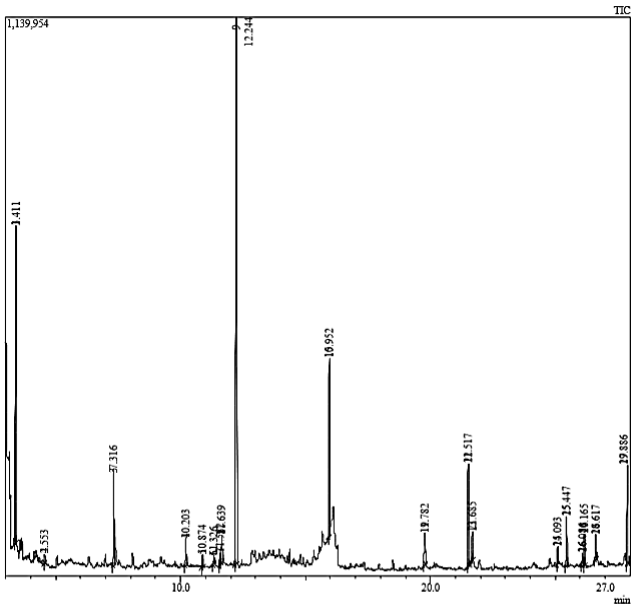

Figure 2: GC-MS chromatogram of the *Ocimum basilicum* ethanol extract

The garlic extract contained high percentage of vitamin E (9.27%), 2,2,5,7,8-pentamethyl-6-chromanol (9.62%), palmitic acid (2.84%) and linoleic Acid (1.70%). Sulfur containing compounds like 3,4-epoxysulfolane were found in lower concentration (0.73%).
A total of 33 bioactive compounds had been qualitatively and quantitatively estimated by GC-MS analysis in seeds ethanol extract of *N. sativa*. Alpha-Linoleic acid (omega 3) was a predominant compound with a percentage of 58.77%. Other major identified compounds include ethyl palmitate (13.30%), methyl linoleate (8.88%), ethyl linoleate (7.35%), and glyceryl monolinoleate (4.50%) as shown in Table 5 and Figure 5.
The GC-chromatogram of *P. nigrum* fruits demonstrated the existence of 45 primary peaks (Table 6, Figure 6). The major components matching the peaks were identified as gamma.-sitosterol (16.28%), piperine (15.56 %), ,1,6-anhydro-4-(3,4-methylene dioxyphenyl methyl amino)-2-O-tosyl-4-deoxy-b-d-glucopyranose (10.56%), caryophyllene (7.99%), (R)-(-)-14-methyl-8-hexadecyn-1-ol (7.78%), linoleic acid (6.30 %), naphthalene, decahydro-1,1-dimethyl- (4.71%), palmitic acid (3.96%), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (3.30%), oleic acid (2.83%), 1-piperidineacetonitrile, .alpha.-(2-phenylethenyl)- (2.22%), (-)-spathulenol (2.13%) and 1-heptatriacotanol (1.29%).
Chemical analysis of the *Z. officinale* ethanol extract showed the presence of 48 phytoconstituents (Table 7, Figure 7) with 7 major peaks corresponding to the following compounds which include: 6-(3,5-dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one (25.171 %), gingerol

(15.61%), zingiberene (12.02%), beta.-sesquiphellandrene (8.88%), decanal (5.98%), curcumene (5.86%), (4-methoxy-phenyl)-(2-nitrocyclohexyl)-methanol (5.60%) and 6-(3,5-dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one (5.09%).

GC-MS analysis (Table 8, Figure 8) of *F. vulgar*e ethanol extract presented 35 identified compounds, cis,cis,cis-7,10,13-hexadecatrienal is a major identified constituent with a percentage of 50.43 %. Other identified peaks are butenoic acid, 2-methyl-, dodecahydro-8-hydroxy-8a-methyl-3,5-bis(methylene)-2-oxonaphtho[2,3-b]furan-4-yl ester, [3ar-[3a.alpha.,4.alpha (13.06%), palmitic acid (3.52%), 2,3,3-Trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone (3.11%) and beta.-monolinolein (3.05%), other components were of the low percentage.

A few studies into the anticancer potential of plants used in Sudanese traditional medicine have been conducted. However, this study describes an *in vitro* anticancer potential of eight Sudanese plants.[45]

Table 3: Major compounds identified by GC-MS analysis in *Linum usitatissimum* ethanol extract

Compound	Retention Time	Peak Percentage (%)
Diethoxymethyl acetate	3.419	8.57
Galactopyranoside, methyl, alpha.-D-	16.854	21.92
Palmitic acid	19.903	8.33
Ethyl palmitate	20.18	1.66
7-Tetradecenal,(Z)	21.936	27.47
Linolenic acid, ethyl ester	22.048	5.28
Monolinolenin,	26.866	11.30
Stigmasterol	28.323	0.65

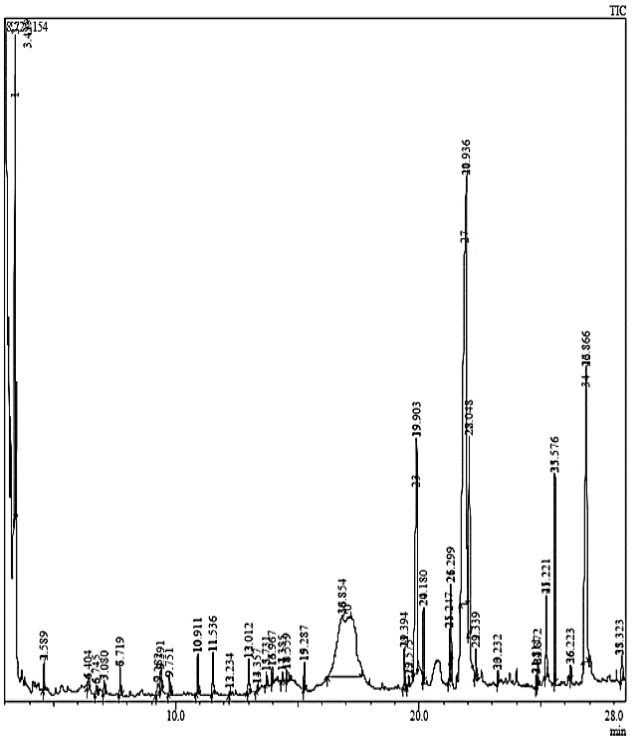

Figure 3: GC-MS chromatogram of the *Linum usitatissimum* ethanol extract.

Table 4: Major compounds identified by GC-MS analysis in *Allium sativum* ethanol extract

Compound	Retention Time	Peak Percentage (%)
Diethoxymethyl acetate	3.452	11.21
3,4-Epoxysulfolane	4.527	0.73
Palmitic acid	19.817	2.84
Linoleic Acid	21.656	1.70
gamma.-Sitosterol	22.291	3.04
Thuja-4(10)-ene-3-ol	23.747	3.16
12-Tricosanone	25.238	19.00
Di-n-octyl phthalate	25.587	27.53
Vitamin E	26.048	9.62
Stigmasta-5,24(28)-dien-3-ol, (3.beta.)-	27.686	1.23
Ergost-5-en-3-ol, (3.beta.)-	27.871	3.67
2,2,5,7,8-Pentamethyl-6-chromanol	28.037	9.27

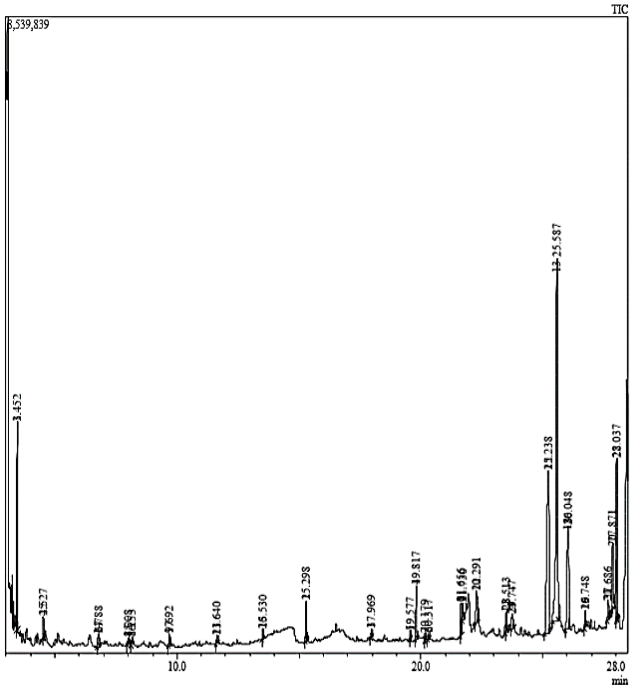

Figure 4: GC-MS chromatogram of the *Allium sativum* ethanol extract.

After 72 hours of treatment, the cytotoxic effects of eight ethanol Sudanese plants ethanol extracts on the two BC cell lines MCF-7 and MDA-MB345 and a normal cell line (HUVEC) were assessed using the MTT experiment, IC_{50} values were calculated using dose-response curves. *M. longifolia* and *O. basilicum* showed promising growth inhibition against MDA-MB-435 with IC_{50} values less than 30 µg/ml, recorded as low as 0.7437 and 16.78 µg/ml, respectively. While, *L. usitatissimum, A. sativum*, and *N. sativa* crude extracts had the strongest significant selective cytotoxic activity against MCF-7 with IC50 values of 2.008, 8.661 and 10.072 µg/ml, respectively. *P. nigrum, Z. officinale*, and *F. vulgar*e ethanol extracts exhibited cytotoxic activities with IC_{50} values higher than 30 µg/ml against both BC cell lines.

None of assayed plants' ethanol extracts showed substantial cytotoxicity against the HUVEC cell line (Table 9).

The ethanol extracts of the tested plants showed qualitative and quantitative compositional variation than those detected in the essential oils and reported in the literature for the same plants. This can be attributed to different factors, including seasonal and maturity differences, geographical location, genetic differences, growth stages, post-harvest drying, storage, and extraction technique.

Some of investigated plant extracts were more cytotoxic to both types of breast cancer cell lines tested than to normal HUVEC cells with a considerable selectivity index (SI) greater than or equal 2.0, whereas, SI = IC_{50} in normal cells/IC_{50} of cancer cells. Moreover, following the American National Cancer Institute's standards, the IC_{50} of crude extracts should not exceed 30 µg/ml, [46,47] only, *O. basilicum* and *M. longifolia* exerted selective significant cytotoxic activities against MDA-MB-435 metastatic TNBC and were less active towards the MCF-7 tumor cell line. While, the ranking order for the best selective antiproliferative ethanol extract against MCF-7 was *L. usitatissimum*, followed by *A. sativum* and *N. sativa*, with comparable IC_{50} values lower than 30 µg/ml.

The chemical profile of *M. longifolia* ethanol extract revealed the presence of a diterpene compound (phytol) in large proportion.

Table 5: Major compounds identified by GC-MS analysis in *Nigella sativa* ethanol extract

Compound	Retention Time	Peak Percentage (%)
Ethyl palmitate	20.442	13.30
Methyl linoleate	21.310	8.88
Alpha linoleic acid	22.766	58.77
Ethyl linoleate	24.262	7.35
Glyceryl monolinoleate	26.961	4.50

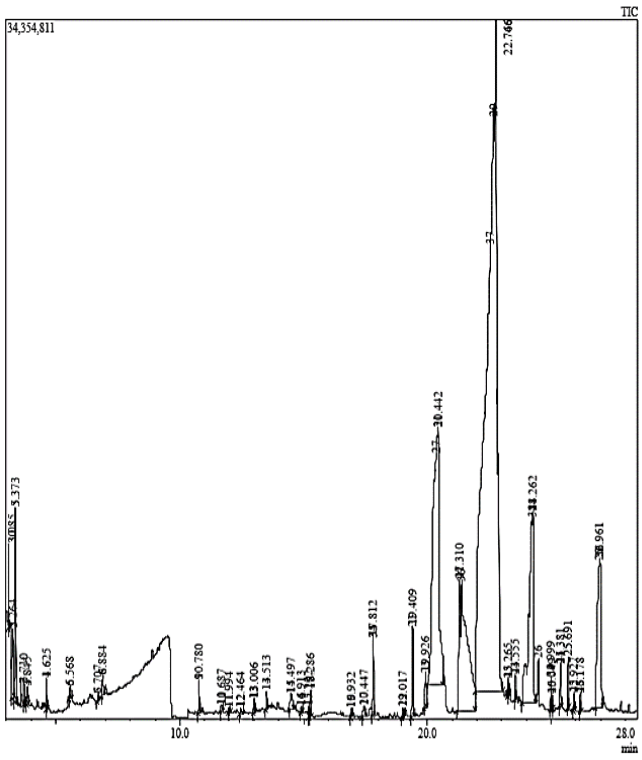

Figure 5: GC-MS chromatogram of the *Nigella sativa* ethanol extract.

The molecular composition of *M. longifolia* ethanol extracts and volatile oils of the same plant varies; the most commonly detected compounds of *M. longifolia* essential oil are piperitone, menthone, piperitenone oxide and carvone. [48] However, the cytotoxic activities of the *M. longifolia* are associated with a higher level of phytol (more than 54%). Phytol is a known diterpene with antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. Sakthivel *et al.*, 2018 reported that phytol exhibited antiproliferative, apoptotic, and antiangiogenic activities against human lung adenocarcinoma cell line.[49,50] Phytol was also found to produce programmed cell death (apoptosis) in the human lymphoid leukemia Molt 4B Cells.[51]

Methyleugenol was detected as a major identified compound in *O. basilicum* leaves ethanol extract. It was also detected as a major constituent in essential oils and methanol extract. [52, 53]

The inhibitory effect of *O. basilicum* on MDA-MB-435 cells could be strongly attributed to their higher contents of methyl eugenol (40.58%) and phytol (6.03%). Methyl eugenol demonstrated cytotoxic and antimigratory effects against the MDA-MB-231 breast cell line.[54]

The ethanol extract of *L. usitatissimum* seed contained fatty acid derivatives. The unsaturated fatty acid derivatives predominantly monolinolenin and linoleic acid ethyl ester were found in higher concentration than saturated fatty acid derivatives represented by palmitic acid. Although, the extraction procedure to extract fatty acids in flax seed in this study differed from other extraction techniques used in other study [55] the percentage of unsaturated fatty acids was still higher than the saturated fatty acids.[55]

L. usitatissimum seed extract contained compounds from various chemical classes the majority of which are fatty acids. Flaxseed's health advantages are thought to be attributed to its fatty acids. Hu *et al.*, (2019) studied the effects of flaxseed extract on human breast cancer MCF-7 cells and found that the flaxseed extract inhibited MCF-7 cell proliferation and promoted apoptosis.[56]

7-Tetradecenal was a major constituent of 27.47% peak area in flaxseed extract and is a liquid lipid reported in the aromatic plant species of coriander as flavour and antioxidant, [57]and it was also identified as a major component in the leaves extract of *Annona muricata* .[58] There is no biological study found using 7-Tetradecenal in any type of application as pure.

Galactopyranoside methyl alpha (21.92%) in flaxseed and its analogs was reported as a potential antimicrobial and antiviral inhibitor of protease enzyme and as antimicrobial and anticancer.[59]

GC-MS profile of garlic (*A. sativum*) ethanol extract is characterized by the presence of di-n-octyl phthalate as a major compound in addition to phytosterol, fatty acid, and vitamin E. Similar bioactive compounds in garlic extract were analyzed previously using HPLC/QQQ/MS.[60] Although fresh garlic is characterized by the presence of sulfur compounds in higher concentrations but GC-MS analysis of ethanol extract showed a lower percentage of sulfur - containing compounds this may be attributed to the different extraction techniques.

A. sativum extract induced cytotoxic effects against MCF-7 but was less active against MDA-MB435 cell lines. The activity of garlic bulb extract could be due to the presence of, vitamin E, 2,2,5,7,8-pentamethyl-6-chromanol, fatty acid (palmitic and linoleic acids), and sterol. The impact of vitamin E (-tocopherol) on breast cancer cell proliferation in estrogen receptor (ER)-positive cells MCF-7 cell was examined, vitamin E showed a dose-dependent decrease of cell proliferation with MCF-7 demonstrating a significant suppression of growth at 100 M vitamin E.[61] In a previous study on anti-carcinogenic effects against breast cancer lines, the ethanol extract of *A. sativum* bulb had cytotoxic, antiproliferative, apoptotic, and antimotility effects on the weakly and strongly metastatic breast cancer cell lines MCF-7 and MDA-MB-231.[62]

The seed of the *N. sativa* plant is a promising source of active compounds like omega 3 that might be used in a variety of clinical contexts. [63]

The antitumor activity of *N. sativa* ethanol extract towards the MCF-7 cell line may be associated with a high level of omega-3. Omega-3 supplements have been shown to lessen the incidence of breast cancer and aid in cancer prevention.[64] Also, Omega-3 fatty acid reduces cell proliferation and causes apoptosis in human breast cancer cells, likely

via inhibiting signal transmission through the Akt/NFB cell survival pathway.[65]

In *P. nigrum* ethanol extract, gamma-sitosterol, and piperine alkaloid appeared as major components. Piperamides and terpenes are the two classes of secondary metabolites in black pepper. [66] *P. nigrum* ethanol extract appeared to be less active towards both breast cancer cell lines in contrast with a study carried out by Buranrat and Boontha who reported that *P.nigrum* extract inhibited the development of human breast cancer cells by causing cell cycle arrest and triggering apoptosis.[67]

The lipids and oleoresins found in ginger's rhizome are divided into two phytochemical classes, phenolics (gingerols) and terpenes (zingiberene and beta.-sesquiphellandrene) compromise major volatile constituents of *Z. officinale* ethanol extract.

The ethanol extract of *Z. officinale* proved to be of low activity against both breast tumor cell lines, the ethanol and aqueous extract of ginger had a dose- and time-dependent anti-proliferative impact on the viability of MCF-7 and MDA MB-231 cells.[68]

Table 6: Major compounds identified by GC-MS analysis in *Piper nigrum* ethanol extract

Compound	Retention Time	Peak Percentage (%)
Caryophyllene	13.031	7.99
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	15.297	3.30
(-)-Spathulenol	15.801	2.13
Naphthalene, decahydro-1,1-dimethyl-	19.330	4.71
Palmitic acid	19.824	3.96
Linoleic acid	21.671	6.30
Oleic acid	21.751	2.83
gamma.-Sitosterol	22.439	16.28
1-Heptatriacotanol	24.793	1.29
1,6-Anhydro-4-(3,4-methylenedioxyphenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose	26.196	10.56
1-Piperidineacetonitrile, .alpha.-(2-phenylethenyl)-	26.266	2.22
Piperine	27.278	15.65
(R)-(-)-14-Methyl-8-hexadecyn-1-ol	27.444	7.78

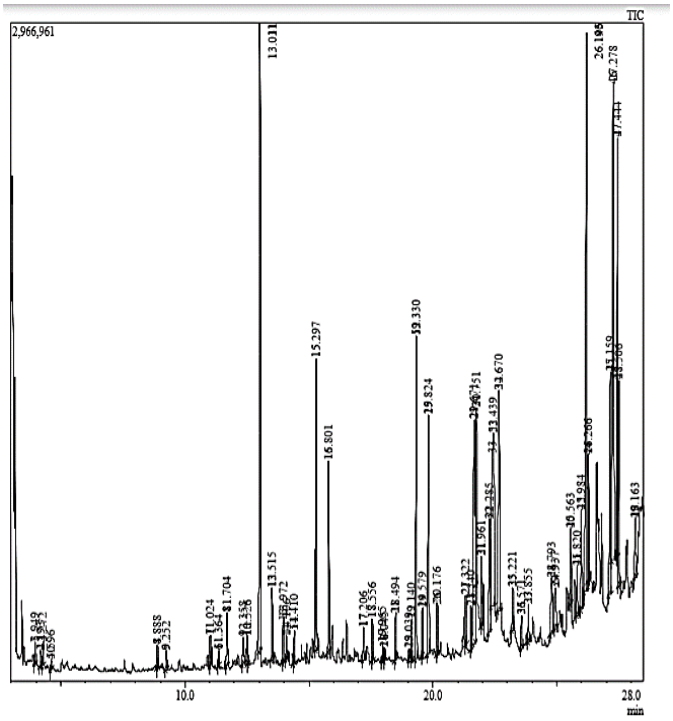

Figure 6: GC-MS chromatogram of the *Piper nigrum* ethanol extract.

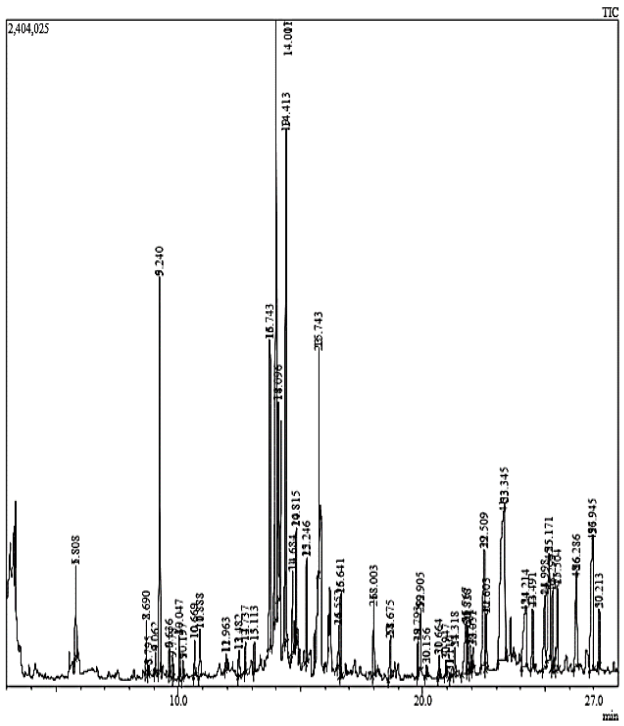

Figure 7: GC-MS chromatogram of the *Zingiber officinale* ethanol extract.

Table 7: Major compounds identified by GC-MS analysis in *Zingiber officinale* ethanol extract

Compound	Retention Time	Peak Percentage (%)
Decanal	9.240	5.98
Curcumene	13.743	5.86
Zingiberene	14.001	12.02
alpha.-Farnesene	14.096	2.76
beta.-Sesquiphellandrene	14.413	8.88
beta.-Elemol	14.684	1.68
trans-Nerolidol	14.815	1.49
(4-Methoxy-phenyl)-(2-nitrocyclohexyl)-methanol	22.509	5.60
Gingerol	23.345	15.61
Carinol	24.998	2.29
6-(3,5-Dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one	25.171	25.171
6-(3,5-Dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one	26.945	5.09

Table 8: Major compounds identified by GC-MS analysis in *Foeniculum vulgar*e ethanol extract

Compound	Retention Time	Peak Percentage (%)
2,3,3-Trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone	18.145	3.11
cis,cis,cis-7,10,13-Hexadecatrienal	21.941	50.43
2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-7,10-dihydroxy-1,1,3,6,9-pentamethyl-4a,7a-epoxy-5	23.578	13.06
Arachidic acid	24.105	1.02
Stearic acid	24.348	1.11
Artemisia ketone	25.518	1.28
beta.-Monolinolein	26.930	3.05

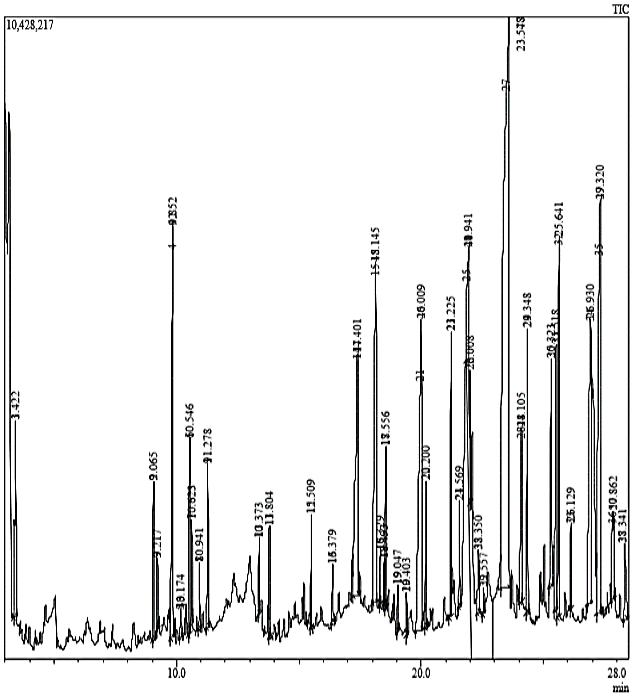

Figure 8: GC-MS chromatogram of the *Foeniculum vulgare* ethanol extract.

GS-MS analyzed components of *F. vulgar*e ethanol extract showed hexadecatrienal as the main compound, components belonged to the isoprenoid class of compounds and trace percentage of sterol, and fatty acid that are significantly different from those demonstrated in other study.[69]

*F. vulgar*e volatile oil rich in trans-anethole which was not detected in this study reported being active against MCF-7 cells. [70] The ethanol extract of this plant was found to exert a week cytotoxic effect against both BC cell lines.

Conclusion

The study was conducted to evaluate and compare the cytotoxic effects of eight Sudanese plants on BC and normal cell lines and to investigate their chemical profile. The ethanol extracts contained different phytomolecules related to several phytochemical classes. The ethanol extracts of *O. basilicum* , *M. longifolia, L. usitatissimum, A. sativum,* and *N. sativa* showed selective cytotoxic effects against different breast tumor cell lines. More *in vivo* research is recommended to investigate the possible anti-carcinogenic benefits of these extracts and their components.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 9: IC_{50} values and selectivity indexes of eight different ethanol extracts against HUVEC, MDA-MB435 and MCF-7 cell lines

Plant name	HUVEC IC50 (µg/ml)	MCF-7		MDA-MB-435	
		IC_{50} (µg/ml)	SI	IC_{50} (µg/ml)	SI
Mentha longifolia	2244.05	60.33 ± 0.19	37.20	0.7437 ± 0.31	>1000
Ocimum basilicum	227.5	48.431 ± 0.17	4.70	16.78 ± 2.47	13.56
Linum usitatissimum	> 1000	2.008 ± 0.18	>1000	203.2 ± 0.14	>1000
Allium sativum	106.7	8.661 ± 0.25	12.32	203.2 ± 0.31	0.53
Nigella sativa	830.9	10.072 ± 0.18	82.50	17959 ± 3.28	0.046
Piper nigrum	275.5	73.664 ± 0.09	3.74	464.9 ± 0.07	0.59
Zingiber officinale	558.39	102.202 ± 0.19	5.46	115.2 ± 0.11	4.85
*Foeniculum vulgar*e	1704.59	142.354 ± 0.11	11.97	92.39 ± 0.02	18.45

Acknowledgments

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