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Phytoconstituents and Cytotoxic Properties of Winged Marigold Extract Against Human Breast Cancer Cells

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Abstract

The chemical analysis results of the alcoholic extract derived from the leaves of Winged Marigold, analyzed through GC-MS, revealed the presence of 53 active compounds belonging to several chemical groups, including organic acids, phenols, flavonoids, terpenoids, and antioxidants. The most prominent compounds are 2,4-dimethylpentanoic acid (15.18%) as the main component, antioxidant compounds such as methyl eugenol, scopa one, oleic acid, anticancer compounds such as psoralen, benzoic acid, compounds toxic to cancer cells such as N, N-Diethyl-5H-chromeno\[4,3-b]pyridin-3-amine, and compounds inducing apoptosis such as Hexa-2,4-diyn-1-ylbenzene. The results of cellular assays on MCF-7 human breast cancer cells showed that the extract had a strong dose-dependent toxic effect, with cell death reaching 87.53% at 100 µg/ml, with a notable reduction in cell viability as the concentration increased. These results indicate that the extract contains bioactive compounds that act through multiple mechanisms, including: Antioxidants that reduce oxidative stress, inhibitors of cancer cell proliferation, and activators of apoptosis. Based on these indicators, the alcoholic extract of Winged marigold can be considered a potential natural therapeutic agent with great promise against breast cancer, given its selective effects on cancer cells and its effectiveness at relatively low concentrations.

Keywords: Winged marigold, GC-MS, antioxidants, MCF-7, cytotoxicity, apoptosis

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Introduction

There are many forms of cancer treatment, including chemotherapy, surgery, and radiotherapy. However, their results remain limited. Despite significant advances in chemotherapy, which is used to stop tumor growth, normal cells are also affected (1). Finding a successful treatment remains a major challenge. Numerous studies have been carried out in the search for a new, safe, and focused treatment for cancer or to alleviate side effects (2,3). The Iraqi desert environment is rich in wild medicinal plants that are used to treat many diseases (4). A considerable number of studies have been undertaken to evaluate the effectiveness of some of these plants and test their anti-inflammatory, antimicrobial, metabolic, and antioxidant activity, and especially anti-cancer using HL-60 and MCF-7

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cell lines (5-8), including colorectal (9), liver, and breast cancer.

The Asteraceae, one of the largest plant families, consists of approximately 1,130 genera and is organized into 13 subfamilies encompassing around 16,200 species (10). Calendula includes more than 25 annual and perennial herbaceous species, with *Calendula tripterocarpa* L. being the most prevalent among them, *Calendula triterpenoida* ruber, *Calendula stellata* Cav, *Calendula suffruticosa* Val, *Calendula officinalis* L., *Calendula field* Linn, and *Calendula* spp. (3).

The winged marigold (*Calendula tripterocarpa* L.), a member of the Asteraceae family, is a rare perennial herbaceous plant native to eastern Saudi Arabia and widespread in desert and semi-arid regions, especially in southern and western Iraq (11), as well as in the dry, sandy environments of the Middle East and North Africa. It is a spring-flowering plant with branched, separate stems, reaching a height of between 10 and 20 cm, characterized by lobed leaves and bright, orange-yellow flowers with a mild fragrance (blooming in autumn to April). The plant life has winged petals; therefore, they are called "winged marigold." This plant is known for its high adaptability to harsh environmental situations, consisting of drought and high temperatures, making it a capacity source of biologically energetic compounds with therapeutic consequences (12,13).

Medicinal vegetation has established its ability to deal with many diseases due to its content of a large spectrum of biologically active secondary metabolites, consisting of alkaloids, antioxidants, flavonoids, terpenoids, phenols, and other chemicals (10). Plants belonging to this genus, such as Calendula tripterocarpa, are generally utilized in conventional remedies for the remedy of diverse illnesses, way to their extensive variety of pharmacological properties, together with the control of burns and minor injuries (10), anti-inflammatory, anti-cancer, antipyretic (14,15), antiviral and antioxidant, antifungal and antiseptic (16).

stated that Calendula flora incorporates many chemicals together with isorhamnetin, rutinkaempferol, isoquercitrin, hyperoside, quercetin 3-O-glucoside, astragalin, quercetin3-O-rutinoside, p-coumaric acid, benzoic acid, chlorogenic acid, and caffeic acid (17), whilst (18) mentioned that the plant consists of violaxanthin, flavoxanthin, β-carotene, auroxanthin, and luteoxanthin. Chemical evaluation of these vegetation additionally found that they include flavonoids, phenols, scopoletin, and quercetin (10). The examiner focused on assessing the phytochemical traits, antioxidant potential, and anticancer properties of the alcoholic extract of the winged marigold plant.

Materials and methods

Samples of winged marigold leaves were collected from the Al-Salman Desert (about 150 kilometers from the center of Al-Muthanna Governorate, southwest of the city of Samawah, near the borders of the Kingdom of Saudi Arabia). Dust and impurities were removed from the plants by washing them thoroughly with plain water, then distilled water, and leaving them to dry at room temperature.

Extraction

The plant leaves were then ground in an electric grinder to obtain a fine powder to increase the surface area of interaction with the solvent. They were then stored in clean, dry plastic bags until use in the laboratory. The extraction steps were carried out in the Laboratory of Drugs and Medicinal Plants of the Pharmacy faculty- Jabir bn Hayan University for Medical and Pharmaceutical Sciences according to the method described by Harborne (1998) using ethanol alcohol at a concentration of 70% as a solvent to extract the active compounds.

Qualitative and Quantitative Analysis of Phytochemicals Using GC-MS

The content of active ingredients in plant leaves was determined using a gas chromatography-mass spectrometry (GC-MASS) instrument, Agilent 5977 A MSD (USA), along with Mass Hunter GC/MS Acquisition Software and Mass Hunter Qualitative Software (USA) were used at the Nahran Omar Field Laboratories of Basora Oil Company. The instrument was configured with an ion source temperature of 230 degrees Celsius, a quadrupole temperature of 150 degrees Celsius, and an interface temperature of 290 degrees Celsius (MSD transfer line). The start time was 4 minutes, and the end time was 35–40 minutes.

Cytotoxicity assays

This assay was conducted in collaboration with the University of Tehran for Medical Sciences. MCF-7 cells were provided by the Cancer Institute of the Tehran University of Medical Sciences and sustained in RPMI-1640 medium, enriched with 10% fetal bovine serum albumin. (FBS), Penicillin was utilized at a concentration of 100 units/mL, alongside streptomycin at a concentration of 100 µg/mL. The cells were resuspended when the cell culture reached approximately 80% confluence, and it is treated with a trypsin-EDTA solution for detachment, passaged twice weekly, and stored at 37°C (11). The toxicity of the plant extract was tested to determine its cytotoxic effects. The viability of methyl thiazolyl tetrazolium (MTT) cells was studied in 96-well plates; cell lines were cultured at a density of 1×10^4 cells per well. After a period of 24 hours, a fully confluent monolayer was obtained by treating the cells with medium containing test components (6.25, 12.5, 25, 50, 100) µg/ml, and 28 µL of 2 mg/ml MTT dye was added. The cells were incubated for 5 hours, and their viability changed into measured after 72 hours of remedy at 37°C. Following the elimination of the MTT answer, 130 µL of 1% dimethyl sulfoxide was added at 37°C for 15 minutes with shaking, and the closing crystals in the wells were cooled. The absorbance turned into decided the usage of a spectrophotometric microplate reader set to a wavelength of 492 nm, and the experiment was done three times. The cellular increase inhibition price (cytotoxicity ratio) is calculated the usage of the following equation: **Cytotoxicity**= a-b/a ×100

To determine cell size under an inverted microscope, 200 µL of cell suspension was added to a 96-well plate at a cell density of 1 × 104 ml-1 and incubated at 37°C for 48 h. The plates were then stained with 50 µL of crystal violet dye and incubated at 37°C for 15 min. The dye was then gently washed with tap water until removed. Cells observed under an inverted microscope were recorded at 40x magnification (19).

Statistical Analysis

The data were statistically analyzed using the unpaired t-test with GraphPad Prism6, as outlined by Jin et al. (2016). The results are expressed as the mean ± standard deviation (SD) based on three measurements, where a P value of less than 0.05 is considered statistically significant.

Results

Phytochemicals of alcoholic extract using the GC-MS technique

Table 1 and Figure 1 reveal the types and quantities of chemical compounds found in the alcoholic extract of Winged Marigold leaves, identified through GC-MS (gas chromatography-mass spectrometry). The analysis identified 53 active compounds, with the extract showing the highest peak area of 15.1819% at 12.712 minutes for the compound 2,4-Dimethylpentanoic acid, which means they may be the main components responsible for the plant's therapeutic effects, while the lowest peak area was 0.1609% at 11.558 minutes for the compound 4-Formyl-1,3(2H)-dihydroimidazole-2-thione (it may have a complementary role or synergistic effects with other compounds.), which indicates that the compounds have varying time of appearance.

The analysis results revealed the presence of various types of these compounds, such as organic acids, phenols, flavonoids, and terpenoids, as well as powerful antioxidants including 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (1.0027%), Methyleugenol (1.6662%), Scoparone (11.56%), Oleic Acid (2.0018%), and n-Hexadecanoic acid (5.5212%). (These compounds are known for their antioxidant properties, are crucial in safeguarding cells against oxidative damage, a major cause of cancer development.). They likewise have anti-inflammatory properties and anti-carcinogenic properties, such as Psoralen, 3-(α,α-dimethylallyl)- (1.6303%), and 4-Vinylbenzene-1,2-diol (10.6018%), Benzoic acid (1.5706%) (These compounds can inhibit the growth of cancer cells by affecting the molecular signals that stimulate cell proliferation, making them useful in the development of cancer treatments). Compounds with toxic effects on cancer cells, such as N, N-Diethyl-5H-chromeno[4,3-b]pyridin-3-amine (3.3837%), Fluconazole (1.9672%), and 2,4-Dimethylpentanoic acid (15.1819%) (These compounds are associated with their cytotoxic effects, as they disrupt the DNA of cancer cells, ultimately causing their death). Compounds that promote apoptosis mechanisms, such as Hexa-2,4-diyn-1-ylbenzene (6.0737%) and 3-Methyldodecanoic acid (6.0234%) (These compounds are involved in activating the mechanisms of programmed cell death in cancer cells, preventing tumor growth and spread).

Cytotoxicity effect of Winged Marigold extract on MCF7cell line

The results of Table 2 and Figure 2 showed that Cytotoxicity % gradually increased with the increase in the extract concentration, until it reached 87.53% at 100 µg/ml, while Viability % decreased clearly in the same reverse pattern, until it reached 12.47% at the highest concentration. At 50 µg/ml, toxicity decreased to 65.12%, while at 6.25 µg/ml it was 31.92%, which means that toxicity decreased at low doses, while the control sample showed no clear effect, which confirms that the changes were caused by the extract and not by other conditions.

It is noted from the data in Table 3 and Figures 3 and 4 that there is a clear inverse relationship between toxicity and cellular vitality, as the concentration of the extract increases, cellular vitality (%) decreases, i.e., the percentage of living cells decreases with increasing concentration. At 100 µg/ml, it was only 12.47%, which means that the extract killed almost all of the cancer cells, and at 50 µg/ml, it was 34.88%, while at a concentration of 6.25 µg/ml, it was recorded at 68.08%, which indicates that a large percentage of cells remained at low concentrations.

Therefore, it can be concluded that the alcoholic extract of Winged Marigold has a strong effect against breast cancer cells at high doses (≥ 25 µg/ml), and that at a concentration of 100 µg/ml, the extract kills a very large percentage of cancer cells. This extract can also be considered a potential candidate for the treatment of breast cancer.

Table (1): Phytochemicals of winged marigold leaf extract

Peak	R.T.	Area Percentage	Library or ID
1	6.436	0.9027	Morpholine
2	8.973	0.4299	1-Methyl-2-piperidinemethanol
3	9.413	0.2298	5-(2-methoxyethylamino)-3H-1,3,4-thiadiazole-2-thione
4	9.822	0.3276	Undecanal dimethyl acetal
5	10.309	0.4967	2-Propen-1-ol
6	10.882	1.0324	1-(1'-Pyrrolidinyl)-2-propanone
7	11.558	0.1609	4-Formyl-1,3(2H)-dihydroimidazole-2-thione
8	11.903	0.4175	1-Propyl-3-piperidinamine, N-acetyl-
9	12.712	15.1819	2,4-Dimethylpentanoic acid
10	12.862	0.5772	2-Propanamine, N-methyl-N-nitroso-
11	12.995	1.0027	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
12	13.301	0.4197	Silane, phenyl-
13	13.474	1.5706	Benzoic acid
14	13.828	0.8307	(-)-trans-Isopiperitenol
15	14.111	0.7912	4-Vinylphenol
16	14.331	0.5957	2-Butenoic acid, 3-methyl-
17	14.55	0.2156	Pyrrolidine, 2-ethyl-1-methyl-
18	14.786	0.2468	Benzenemethanol, 4-hydroxy-
19	15.053	0.5455	Pyrazine, 2-ethyl-5-methyl-
20	15.886	0.2929	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-
21	16.051	0.3774	Acrylamide, N-(2-pentyl)-N-(2-ethylhexyl)-
22	16.514	0.9854	Methyleugenol
23	16.687	1.6662	Methyleugenol
24	17.245	10.6018	4-Vinylbenzene-1,2-diol
25	17.952	6.0737	Hexa-2,4-diyn-1-ylbenzene
26	18.203	1.6574	2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one
27	18.47	0.1921	Spiro[1,3-dioxolane-2,2'(1'H)-naphthalene], octahydro-, trans-
28	19.02	4.8931	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-
29	19.719	1.5229	Benzene, 2,4-pentadiynyl-
30	19.845	2.5279	2-Amino-4-tert-amylphenol
31	20.081	6.0234	3-Methyldodecanoic acid
32	20.827	1.4992	Tetradecanoic acid
33	20.968	1.172	Hexylamine, N,N-di(allyl)-
34	21.73	0.8086	2-Pentadecanone, 6,10,14-trimethyl-
35	22.076	0.3764	Cyclotetradecane
36	22.202	0.4476	4H-Pyran-4-one, 2-methyl-6-phenyl-
37	22.39	0.7053	Methanol, TBDMS derivative
38	22.602	0.7435	1H-Cyclopenta(b)quinoline, 2,3,5,6,7,8-hexahydro-9-amino-
39	22.901	5.5212	n-Hexadecanoic acid
40	23.246	11.56	Scoparone

41	23.867	0.4674	Vasicinone
42	24.04	0.6448	1-Octadecene
43	24.33	0.5933	Phytol
44	24.55	2.0018	Oleic Acid
45	24.825	1.9672	Fluconazole
46	25.634	0.3683	(1R,2S,4r)-4-((E)-prop-1-en-1-yl)cyclopentane-1,2-diol
47	26.066	1.6303	Psoralen, 3-(.alpha.,.alpha.-dimethylallyl)-
48	26.475	3.3837	N,N-Diethyl-5H-chromeno[4,3-b]pyridin-3-amine
49	27.669	0.2741	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
50	27.999	0.477	Bis(2-ethylhexyl) phthalate
51	28.25	0.2173	Piperazine, 1,4-di(carbocyclohexyl)-
52	28.721	0.6973	4'-(tert-Butyl)-2-hydroxystilbene
53	29.004	1.6545	9H-xanthen-9-one, 1-(1,1-dimethylethyl)-

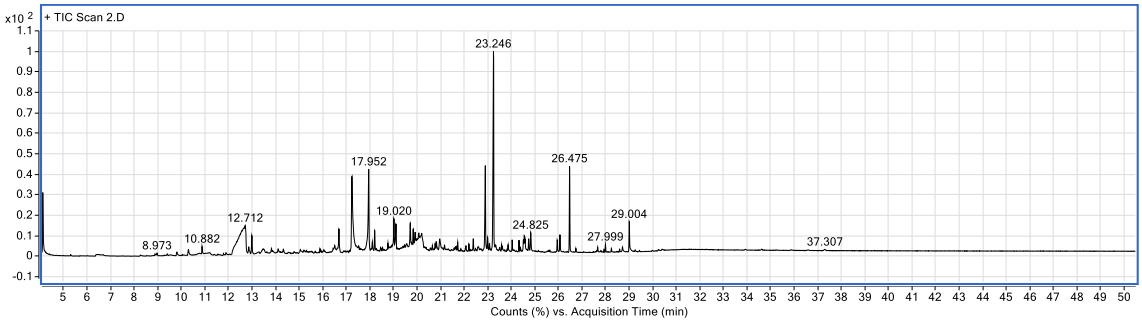

Figure 1: Analysis of GC-MS of the alcoholic extract of the Winged Marigold plant

Table (2): Effect of alcoholic extract of Winged Marigold on MCF7cell line						
	Concentration	**R1**	**R2**	**R3**	**Average**	**Cytotoxicity %**
1	**100µg/ml**	0.11	0.29	0.19	**0.197**	**87.53**
2	**50µg/ml**	0.58	0.61	0.46	**0.55**	**65.12**
3	**25µg/ml**	0.61	0.68	0.67	**0.653**	**58.56**
4	**12.5µg/ml**	0.82	0.87	0.9	**0.863**	**45.24**
5	**6.25µg/ml**	0.99	1.21	1.02	**1.073**	**31.92**
6	**control**	1.52	1.64	1.57	**1.577**	

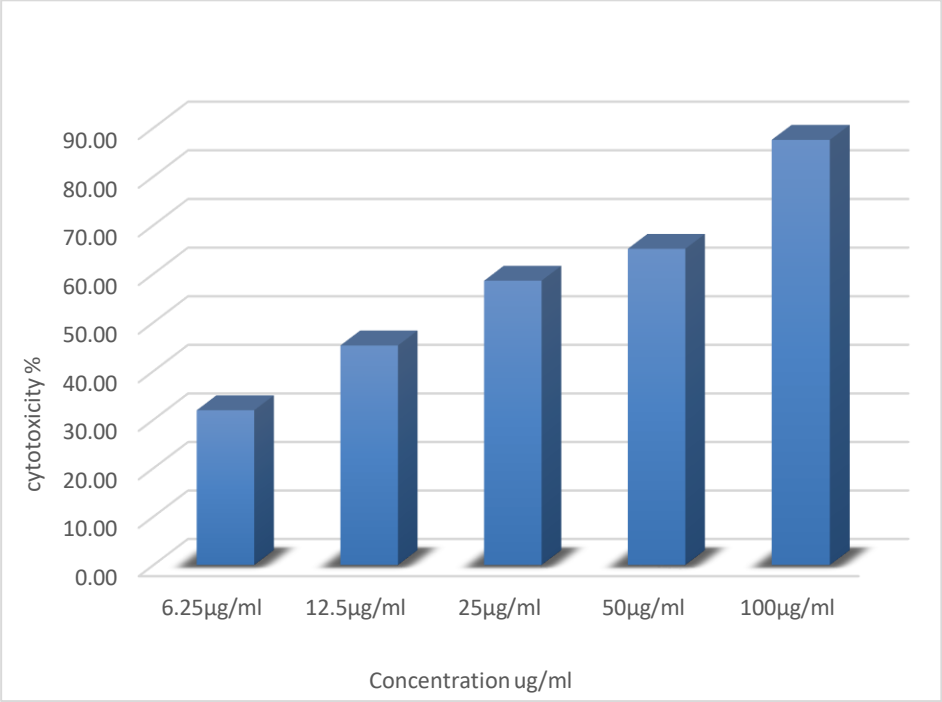

Figure (2): Cytotoxicity effect of the Alcoholic extract of Winged Marigold on the MCF7cell line(breast cancer)

Table (3): Viability and dose response

	Concentration	Viability
1	6.25µg/ml	68.08
2	12.5µg/ml	54.76
3	25µg/ml	41.44
4	50µg/ml	34.88
5	100µg/ml	12.47

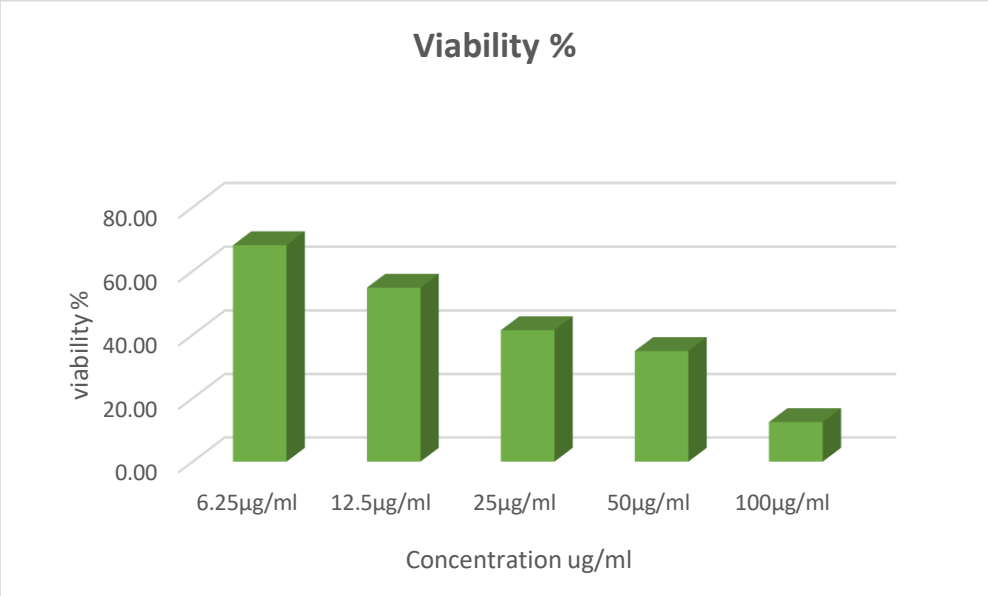

Figure (3): Viability chart after exposure to the alcoholic extract of the Winged Marigold plant

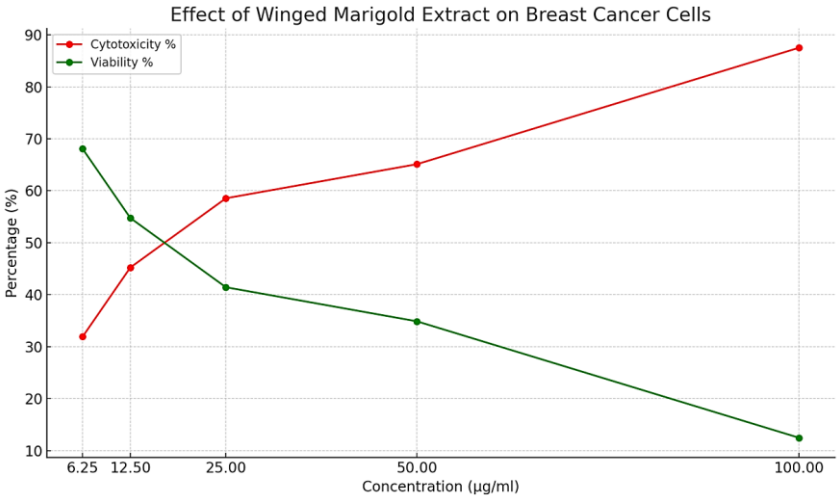

Figure (4): Combined chart diagram comparing the inverse relationship between Cytotoxicity and Viability

Discussion

The GC-MS approach serves as an effective approach for both qualitative and quantitative evaluation and identity of a wide range of phytochemical compounds (20). Phenols, along with methyl eugenol, are recognized for their antioxidant and antimicrobial features (21). Flavonoids and terpenoids, including α-farnesene, have anti-inflammatory functions, and phthalate compounds, including dibutyl phthalate and bis(2-ethylhexyl) phthalate, are not unusual in essential oils and are used within the clinical and pharmaceutical industries (22). Methyl eugenol, scoparone, and oleic acid are acknowledged for his or her antioxidant properties, playing a vital role in safeguarding cells in opposition to oxidative harm, a prime motive of cancer development (23). These compounds have anti-inflammatory and anti-cancer features, along with psoralen, 3-(α,α-dimethylallyl)-, and benzoic acid, which might be able to inhibit the growth of most cancer cells with the aid of affecting the molecular alerts that stimulate mobile proliferation, making them beneficial in the improvement of most cancer treatments (24). Compounds that promote apoptosis mechanisms, including Hexa-2,4-diyn-1-ylbenzene and three-methyldodecanoic acid (20). The promising role of plant extracts in fighting breast cancer stems from their selective toxic consequences on cancer cells. This impact is linked to the presence of various institutions of biologically active compounds with confirmed potential to steer cellular boom pathways, induce apoptosis, and suppress the increase of breast cancer cells (25). Antioxidants are important in safeguarding cells from oxidative stress, a prime component in the development of cancer.

Conflict of interest: NIL

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