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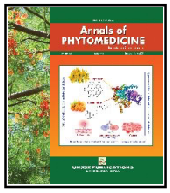

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Evaluation of antioxidant and anticancer activities of a herbal formulation on breast cancer cell line (MCF-7)

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Abstract

With around 2.26 million documented cases, breast cancer ranks among the most widespread forms of cancer worldwide. Surgical and chemotherapeutic interventions have various adverse consequences. The high costs associated with certain medications used in chemotherapy, along with their limited efficacy, contributes to the ineffectiveness of treatment under standard regimens. There is an increasing need for safe and effective methods to handle cancer in general, with a specific focus on breast cancer. Unani physicians provided a comprehensive description of cancer (Saratan) and put forth multiple treatment plans for its control. The Unani classical text has descriptions of compositions containing multiple ingredients that exhibit anticancer properties in humans. A herbal formulation called "Matbukh Aftimoon", consisting of fourteen drugs, was used to study its antioxidant and anticancer effects. The DPPH assay was employed to assess its antioxidant activity, while the MTT assay was used to evaluate its anticancer properties on MCF-7 breast cancer cells. The formulation was standardised and high-performance thin layer chromatography (HPTLC) was conducted to create a fingerprint for future utilisation of this formulation. The DPPH assay results indicated that the aqueous extract exhibited the highest activity with an IC_{50} value of 31.14 µg/ml. In comparison, the methanolic extract and hydroethanolic extract had IC_{50} values of 56 µg/ml and 58 µg/ml, respectively. Ascorbic acid, on the other hand, displayed an IC_{50} value of 21 µg/ml. The MTT assay demonstrated that the methanolic, hydroethanolic, and aqueous extracts exhibited significant activity, with IC_{50} values of 33 µg/ml, 50 µg/ml, and 53 µg/ml, respectively, compared to paclitaxel with an IC_{50} value of 18µg/ml. This study offers valuable information regarding the potential use of test drug as a therapy for breast cancer.

1. Introduction

Cancer is indeed one of the leading causes of death globally. According to statistics from reputable sources like the WHO, cancer is a significant public health challenge, responsible for millions of deaths each year. In 2020 alone, it accounted for nearly 10 million deaths worldwide. These numbers underscore the importance of ongoing research, prevention efforts, and improved access to quality healthcare in the fight against cancer (Ferlay *et al.,* 2021). In 2020, breast cancer stood out as the most prevalent cancer globally, with around 2.26 million reported cases, following that lung carcinoma 2.21 million, and rectum and colon cancer 1.93 million cases. According to data by WHO, there were 2.3 million addition of breast cancer among women in 2020, resulting in 685,000 fatalities globally. A total of 7.8 million women were diagnosed with breast cancer in 2020. Women after puberty may be affected by breast cancer irrespective of age and geographical barrier, with rates typically rising with age (Hyuna *et al.,* 2021). Forecasts indicate that by 2040, the breast cancer incidence will increase 40% while death due to cancer will increase by 50% (Arnold *et al.,* 2022).

The term "Saratan" comes from Arabic and means "crab". Its origins can be traced back to the ancient Greek word καρκίνος (karkinos), also meaning "crab". Galen (Jalinus), a notable figure in Unani medicine (131-200 AD), drew a comparison between a disease and a crab, describing how the tendrils of the illness extend like the claws of a crab. This analogy underscores the complex nature of the disease, with its extensions resembling the claws of a crab, a concept that has persisted throughout the history of medical understanding (Aslam *et al.,* 1981). According to Unani nomenclature, Waram-o-Salâba is characterised by severe inflammation, lesions, and branches filled with a melancholy humour (Madda-i-sawdâwî) (Maseehi, 1995). This condition typically develops in individuals who are overweight and have a flabby build, which contributes to a higher incidence of cancer, especially in hollow organs where the causative melancholy humour can easily accumulate, such as the breast, lungs, cervix, oral cavity, and uterus (Maseehi, 1995; Ibn Rushd, 1980; Sina, 2010). Cancer manifests as a firm, warm inflammation deeply rooted within the tissue, accompanied by tenderness and dryness. The pain associated with it intensifies due to pressure from the mass. Initially, the growth may be small, like a pea, but it can grow to the size of a watermelon. If the condition begins with unbearable pain, it is considered untreatable. However, if the growth progresses without

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significant pain, treatment may help to regress its advancement (Sina, 2010; Hubal, 2005).

In Unani medical tradition, onset and progression of cancer are shaped by various factors, notably the overproduction of black bile (Sawdâ) and its irregular transformations. The transformations occur in specific patterns: overproduction of typical black bile, conversion of typical black bile into atypical black bile, formation of black bile through the burning of blood, creation of black bile from the combustion of phlegm (Balgham), and development of black bile by other processes. Furthermore, black bile can also result from the burning of yellow bile (Safra), specifically known as bilious melanchole (Mirra-i-sawdâ) (Razi, 1991; Arzani, 2020; Qamri, 2008). Unani Physicians have also identified cancer as a melancholic inflammation (Warm-i-sawdâwî), characterized by a melanotic swelling. This arises from an imbalance in the combustion process of yellow bile alone or both phlegm and yellow bile within the body (Maseehi, 1995; Zuhr, 1986).

According to Galen, purging the body of unhealthy humours involves employing concoctive to black bile (Mundij-i-sawdâ') and purgative for black bile (Mushil-i-sawdâ') (Razi, 1997). Treatment involves the use of both single and compound drugs. Among the commonly utilized Unani medications are Aftimoon (*Cuscuta reflexa* Roxb.), Turbud (*Operculina turpethum* (L.) Silva Manso), Bisfayej (*Polypodium vulgare* L.), Sadabahar (*Catharanthus roseus* (L.) G. Don), Kutki (*Picrorhiza kurroa* Royle. ex Benth.), and others. These individual drugs have demonstrated potential anticancer effects in scientific studies. Moreover, they have been employed for various ailments over a significant period and continue to be in use today (Razi, 1991; Mannan *et al.,* 2021).

A traditional Unani blend herbal formulation named Matbukh Aftimoon (MA) contains fourteen ingredients, many of which possess scientifically validated antioxidant and anticancer properties. This formulation is beneficial in treating melancholic diseases (Sawdâwî amrâd) and functions as concoctive to black bile (Mundij-i-sawdâ'), purgative for black bile (Mushil-i-sawdâ) and purgative for phlegm (Mushil-i-balgham) (Table 1).

Table 1: Ingredients of the herbal formulation

S.No.	Name	Botanical name	Part used	Quantity
1.	Aftimoon	*Cuscuta reflexa* Roxb	Whole plant	52 g
2.	Alu bukhara	*Prunus domestica* L.	Fruit	50 g
3.	Maweez munaqqa	*Vitis vinifera* L.	Fruit	35 g
4.	Amla	*Phyllanthus emblica* L.	Fruit	17.5 g
5.	Post balela	*Terminalia bellirica* (Gaertn.) Roxb.	Pericarp of mature fruit	17.5 g
6.	Badranjboya	*Melissa officinalis* L.	Whole plant	14 g
7.	Ustukhuddus	*Lavandula stoechas* L.	Whole plant	14 g
8.	Gaozaban	*Borago officinalis* L.	Leaves	14 g
9.	Ghafis	*Gentiana olivieri* Griseb.	Flower	14 g
10.	Halela siyah	*Terminalia chebula* Retz.	Immature fruit	12.5 g
11.	Post halela kabuli	*Terminalia chebula* Retz.	Pericarp of mature fruit	12.5 g
12.	Post halela zard	*Terminalia chebula* Retz.	Pericarp of semi mature fruit	12.5 g
13.	Bisfayej	*Polypodium vulgare* L.	Rhizomes	10.5 g
14.	Ghariqoon	*Agaricus albus* L.	Fruiting body	3.5 g

To establish quality standards, authenticity, antioxidant activity, and anticancer potential using contemporary analytical methods, this classical Unani compound herbal formulation, as outlined in Unani pharmacopoeia like "Ilaj-ul-Amraz" and "Al Qarabadeen" has been identified and chosen for investigation (Kabiruddin, 2006; Khan, 2005).

Modern cancer treatments include chemotherapy and radiation, which have significant adverse effects. Most chemotherapy medications target quickly dividing cells, affecting bone marrow, the gastrointestinal (GI) system, and hair follicles. These drugs cause myelosuppression, nausea, vomiting, GI problems, and reduced blood cell formation. These medicines can cause myelosuppression, nausea, vomiting, GI problems, mucositis, and alopecia, reproductive disorders like sterility, infertility, and infusion reactions. Furthermore, there is a greater vulnerability to infections as a result of compromised immune function (immunosuppression) (Amjad *et al.,* 2024).

2. Materials and Methods

2.1 Crude drugs procurement and authentication

The crude drugs were authenticated by the botanist at the Institute and Voucher Specimen No. for Halela kabuli, halela zard, halela siyah, balela, amla, ustukhuddus, maweez munaqqa, gaozaban, badranjboya, ghafis, bisfayej, ghariqoon, alu bukhara and aftimoon were assigned as SMPU/CRI-Hyd-15122, 15123, 15124, 15125, 15126, 15127, 15128, 15129, 15130, 15131, 15132, 15133, 15134 and 15135, respectively.

2.1.1 Preparation of test formulation

The study formulation "MA" was prepared in the GMP certified pharmacy of the Institute, according to the standard guidelines mentioned in Unani pharmacopoeia, Al Qarabadeen and Ilaj-ul-Amraz. The individual ingredients were pulverised separately and

then mixed to get final composition. Aqueous, hydroethanolic (1:1), and methanolic extract was prepared by combining 10 g of powdered MA with 100 ml of the corresponding solvent. The mixture was stirred at a speed of 50 rpm in a rotatory shaker incubator at a temperature of 37°C. Subsequently, the mixture underwent filtration and drying using a rotatory vacuum evaporator. The resulting product was then stored in a desiccator.

2.2 Organoleptic parameters

Organoleptic parameters like texture, colour, odour, taste was evaluated to differentiate them from other plants (Singh *et al.,* 2023; QSIMP, 2003; UPI, 2008).

2.2.1 Microscopic evaluation

All powders were stained separately with phloroglucinol 1% and conc. HCl for identification of lignified cells and tissues, H_2SO_4 to detect calcium oxalate crystals, iodine solution was used to find starch granules, Sudan red-G for cuticular cell walls, and Sudan red G in acetic acid and ethanol for the presence of essential oils, fats and resins and observed under microscope (QSIMP, 2003).

2.3 Physicochemical evaluation

The parameters included measurements of weight loss upon drying, pH of 1% and 10% aqueous suspensions, aqueous, alcoholic, hexane, and chloroform extract (UPI, 2008).

2.3.1 Qualitative analysis of phytoconstituent of the extracts

The aqueous, methanolic and chloroform extracts of MA were subjected to test the presence of therapeutically active chemicals like alkaloids, glycosides, fixed oil, *etc*., by using standard colour reactions tests (Chaithra *et al.,* 2022; Venkatesham *et al.,* 2021; Beg *et al.,* 2022).

2.4 TLC, HPTLC analysis and phytochemicals screening

The 5 g MA was extracted in 100 ml of ethanol and chloroform in conical flask for 6 h and filtered and dried to 5 ml. The concentrated extract was used for TLC and HPTLC on Silica Gel 60 F254 (Merck) plate by using toluene, ethyl acetate, and methanol (9:1:0.5) as mobile phase. TLC plates were detected in UV-Vis chamber at 366 nm, and 254 nm, and 1% vanillin sulphuric acid was used as detection spray. HPTLC was done by fully automatic CAMAG HPTLC instrument (Singh *et al.,* 2023).

2.5 Preparation of extract of MA

Methanolic, hydroethanolic (1:1) and aqueous extract of MA, obtained by continuous extraction by Soxhlet's apparatus and dried in lyophilizer was used to test antioxidant and anticancer activity of MA. The dried extract was dissolved in dimethyl sulfoxide (DMSO) to prepare 10 mg/ml stock solution (Kumar *et al.,* 2023).

2.6 Antioxidant assay

The DPPH assay was conducted to assess the antioxidant capacity. This strategy was evaluated using the approach described by More *et al.*, 2020, with minor adjustments. In summary, a portion of 100 µl of 0.1 mM DPPH in methanol was mixed with 50 µl of MA extracts of in a micro test plate. The plate was placed in a dark environment and incubated for 30 min to allow for the desired response. The optical density of the plate was then taken using a TECAN multimode reader at a wavelength of 517 nm. Ascorbic acid was used as control for the experiment (More *et al.*, 2020).

The free radical scavenging activity (% inhibition) was calculate using following formula:

$$\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

2.7 Cytotoxicity Assay

Estrogen receptor (ER)-positive MCF-7 were received from National Centre for Cell Science, Pune, India. MCF-7 breast cancer cells were cultured in T-25 and T-75 flasks, using Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, 1% 200 mM L-glutamine, and 0.5% antibiotic-antimycotic solution. These cultures were maintained in a CO_2 incubator with conditions set at 95% air, 100% relative humidity, 5% CO_2, and a temperature of 37°C. Passaging of cultures occurred every 5 days, and the culture media was refreshed three times per week. Trypan Blue method was used to count of viable cells with the help of haemocytometer (Lee *et al.,* 2010).

2.7.1 MTT Assay

In a 96-well flat-bottom plate, 200 µl of media was dispensed into each well and seeded with 5000 MCF-7 cells per well. The cells were allowed to adhere overnight, following which various concentrations of aqueous, hexane, and methanol extracts (0.1, 1, 10 and 100 ig/ml) of the MA were added to the respective wells. After a 24 h incubation at 95% air, 100% relative humidity, 5% CO_2 at 37°C, 100 µl of MTT reagent (2.5 mg MTT in 10 ml phosphate buffer saline) was introduced into each well. After an additional 4 hours of incubation, 100 µl of DMSO solution was applied to each well to dissolve the MTT crystals. Subsequently, optical density was measured using a TECAN multimode reader at a wavelength of 590 nm. Paclitaxel was employed as the standard drug. Each concentration was repeated in sets of three, and data was obtained through three measurements. The cells that were not treated were utilised as the control to measure viability, representing 100%. The results are displayed as percentages indicating the level of viability. The effectiveness of inhibiting cell growth for each extract will be indicated by its IC_{50} value (Bahuguna *et al.,* 2017).

% Inhibition of extract against cell line was measured by formula given bellow:

$$\% \text{ Viability} = \frac{\text{absorbance of test sample}}{\text{absorbance of control (untreated cells)}} \times 100$$

% Cell inhibition = 100 – % Cell viability

2.8 Statistical analysis

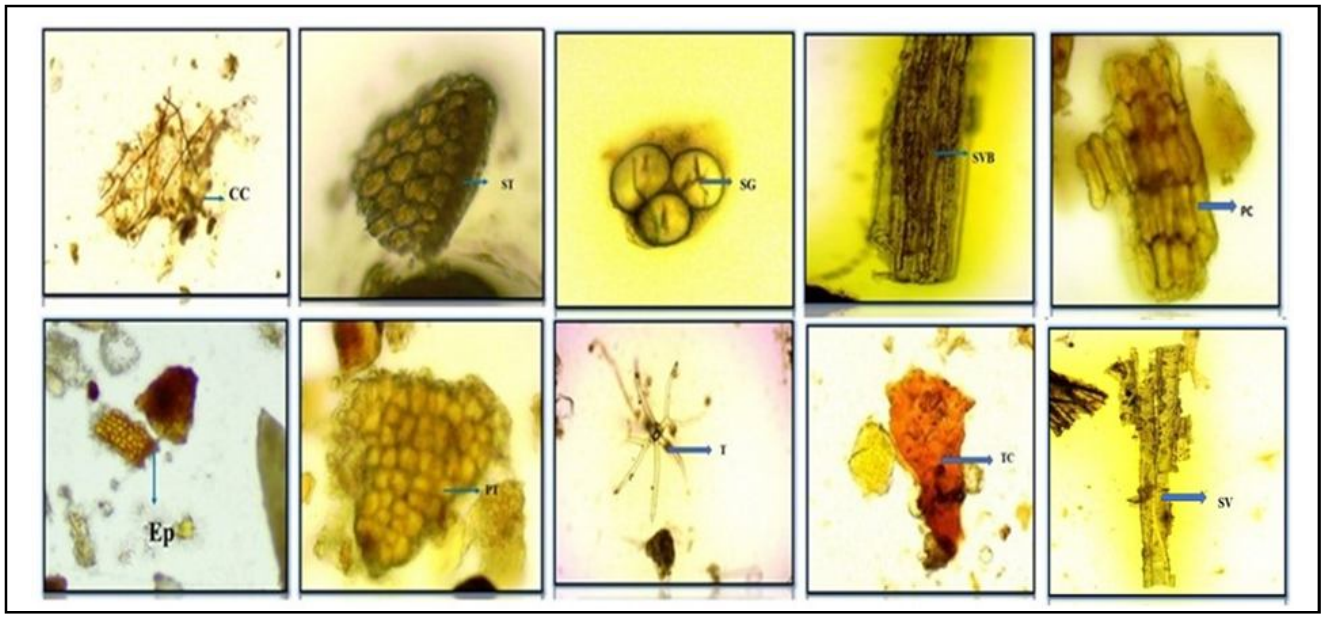
The statistical calculation was conducted by the help of Graph Pad Prism 5.0 and ANOVA by using open epi software. The values of $p \leq 0.05$ was considered as statistically significant.

3. Results

3.1 Microscopic evaluation

The microscopic characters of MA powder are shown in Figure 1. It consists of multicellular, uniseriate, non-glandular trichomes together with glandular trichomes with globular unicellular head. Fragments

of parenchymatous cells with inter cellular spaces, palisade and spongy tissues, thin-walled cells of epidermis, strands of vascular bundles, starch grains are present, scalariform vessels are very clear, tannin cells and layers of cork cells were observed.



CC (Cork cell), ST (spongy tissue), SG (starch grain), SVB (strands of vascular bundles), PC (parenchymatous cell), EP (epidermis), PT (palisade tissue), T (trichomes), TC (tannins cells), SC (scalariform vessels).

Figure 1: Microscopy of the powder form of MA.

3.2 Physicochemical analysis

The average values of alcohol soluble extracts for batch-I, batch-II and batch-III were determined to be 21.1187 ± 0.03, 21.1161 ± 0.01 and 21.1137 ± 0.04 respectively. The water soluble extracts were found to be 50.8688 ± 0.07, 50.8811 ± 0.05 and 50.906 ± 0.08 for all batches, respectively. The mean values of Hexane soluble extract were found to be as 0.7638 ± 0.004, 0.7610 ± 0.009 and 0.7668 ± 0.007 for all three batches, respectively. The mean values of chloroform soluble extract of MA were found to be as 2.5530 ± 0.12, 2.5503 ± 0.11 and 2.5209 ± 0.10 for all batches, respectively. The pH of 1% aqueous suspension was determined to be 4.14 ± 0.06, 4.19 ± 0.08 and 4.17 ± 0.03, whereas pH of 5% aqueous suspension was found 4.44 ± 0.07 and 4.4 ± 0.04 and 4.41 ± 0.15 for three batches. The average loss of weight on drying at 105°C were found as 8.5927 ± 0.10, 8.2736 ± 0.17 and 8.4225 ± 0.06 for all batches, respectively (Table 2).

Table 2: Physicochemical analysis of MA

S.No.	Parameters	Values (I)	Values (II)	Values (III)
1.	Alcohol soluble extract (% w/w)	21.1187 ± 0.03	21.1161 ± 0.01	21.1137 ± 0.01
2.	Water soluble extract (% w/w)	50.8688 ± 0.07	50.8811 ± 0.05	50.906 ± 0.08
3.	Hexane soluble extract (% w/w)	0.7638 ± 0.004	0.7610 ± 0.009	0.7668 ± 0.007
4.	Chloroform soluble extract (% w/w)	2.5530 ± 0.12	2.5503 ± 0.11	2.5209 ± 0.10
5.	pH of 1% aqueous solution	4.14 ± 0.06	4.19 ± 0.08	4.17 ± 0.03
6.	pH of 5% aqueous solution	4.44 ± 0.07	4.40 ± 0.04	4.41 ± 0.15
7.	Loss in weight on drying at 105°C	8.5927 ± 0.10	8.2736 ± 0.17	8.4225 ± 0.06

3.3 Qualitative phytochemical analysis

The results of the colour reaction for the presence of secondary metabolites are depicted in Table 3. It has been observed that all extracts showed good contents of chemical constituents. The results reveal the presence of therapeutically active secondary metabolites like alkaloids, glycoside, carbohydrate, flavonoids, saponins, tannins, protein, phenols and terpenoids, *etc*. Ethanolic extract showed all chemical constituents except steroids while glycoside, saponin and steroids were absent in water extract. While chloroform extract was devoid of carbohydrates, flavonoids, steroids, protein and phenol.

Table 3: Chemical constituents of MA

S.No.	Constituents	Results		
		Ethanolic extract	Aqueous extract	Chloroform extract
1.	Alkaloids	+++	+++	++
2.	Glycosides	++	++	–
3.	Carbohydrate	+++	+	-
4.	Flavonoids	+++	++	-
5.	Saponins	++	-	+
6.	Steroids	-	-	-
7.	Tannin	++	+++	++
8.	Proteins	+	+	-
9.	Phenols	+++	+	-
10.	Terpenoids	+	+	+

3.4 High performance thin layer chromatography of MA

HPTLC of chloroform extract under UV-254 nm showed five major peaks at R_f values 0.007, 0.286, 0.456, 0.790 and 0.851 while under UV-366 nm shows four major peaks of R_f 0.007, 0.117, 0.207 and 0.755. Eight spots with Rf values 0.031, 0.060, 0.090, 0.301, 0.426, 0.489, 0.653 and 0.735 were seen after derivatized with 1% vanillin-sulphuric acid in visible light (Figure 2).

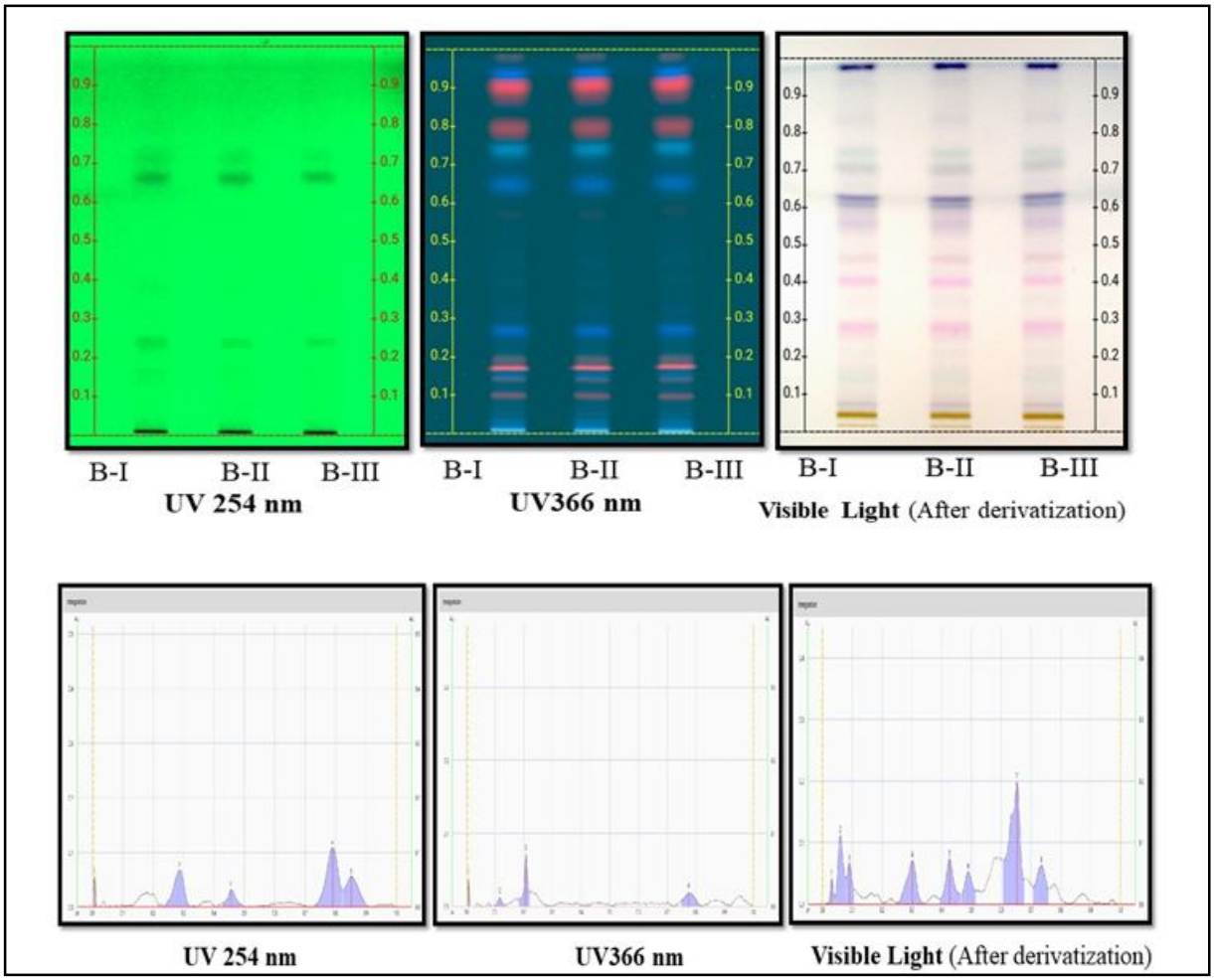


Figure 2: Rf values of HPTLC of chloroform extract of MA.

HPTLC fingerprint profile of alcoholic extract under UV-254 nm showed eight major peaks at Rf values 0.007, 0.029, 0.136, 0.253, 0.489, 0.539, 0.587 and 0.649 while five major peaks of R_f values 0.004, 0.026, 0.127, 0.527 and 0.676 under UV-366 nm. After derivatization with 1% vanillin-sulphuric acid five spots at Rf values 0.021, 0.543, 0.603, 0.660 and 0.899 were seen (Figure 3).

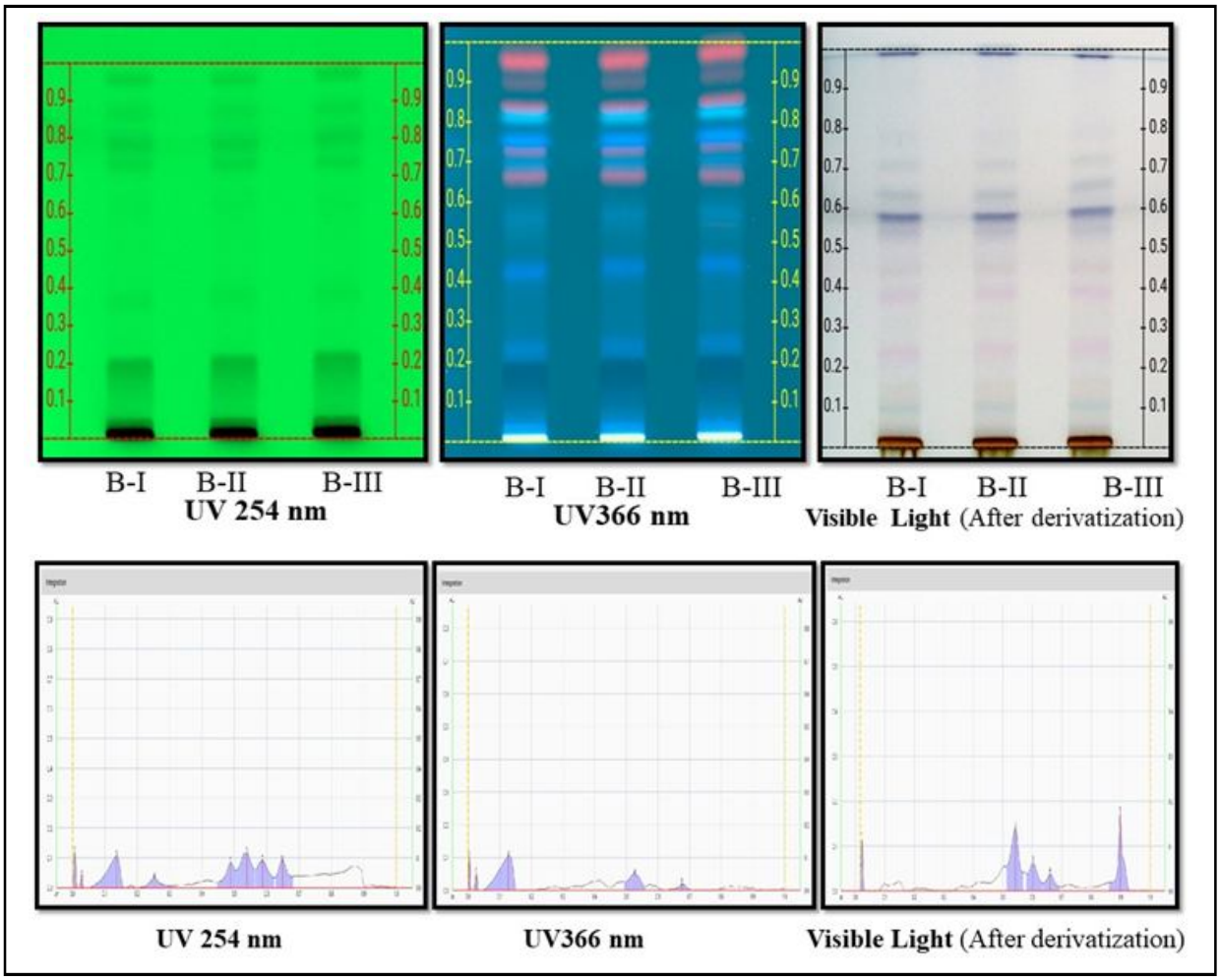


Figure 3: Rf values of HPTLC of alcoholic extract of MA.

3.5 DPPH radical scavenging activity

The antioxidant activity of all extracts showed dose dependant results increasing with higher concentration. Result showed highest activity in aqueous extract (IC_{50} value 31.14 µg/ml), methanolic extract (IC_{50} value 56 µg/ml) and then hydroethanolic extract (IC_{50} value 58 µg/ml), when equated with Ascorbic acid (IC_{50} value 21 µg/ml) (Tables 4,5 and Figure 4).

Table 4: Antioxidant activity of MA by DPPH assay

Concentrations (µg)	Aqueous extract	Hydroethanolic extract	Methanolic extract	Ascorbic acid
0.1	25.77	1.02	15.6	28
1	39.75	10.15	25.47	44
10	51.63	33.95	45.23	59
100	77.3	75.29	64	79

Table 5: IC_{50} of different extracts of MA using DPPH assay

Antioxidant activity of MA				
Parameter	Aqueous extract	Methanolic extract	Hydroethanolic extract	Ascorbic acid
IC_{50} value (µg/ml)	31	56	58	21

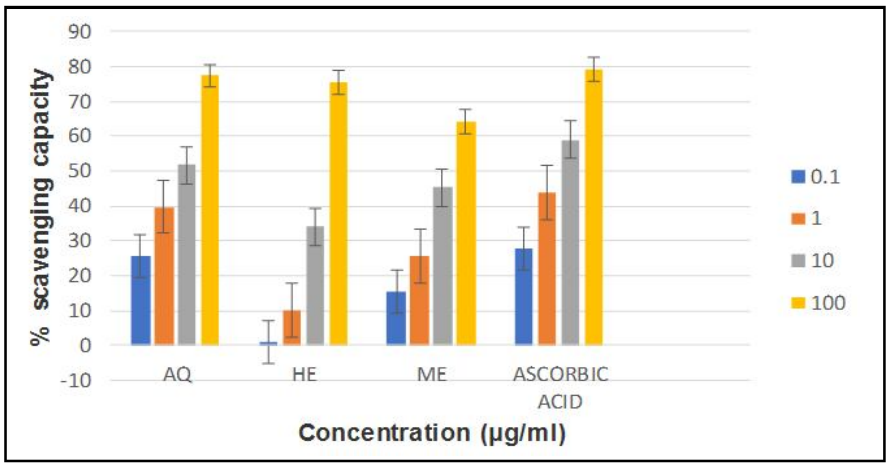


Figure 4: Antioxidant activity of MA.

3.6 MTT assay

The cytotoxicity activity was evaluated at different concentrations extending 0.1 to 100 µg/ml, and subsequently IC_{50} was calculated. Positive control Paclitaxel has showed an IC_{50} value of 18 µg/ml while methanol, hydroethanol and aqueous extracts of MA exhibited IC_{50} value of 33 µg/ml, 50 µg/ml and 53 µg/ml, respectively. The result showed that the activity was dose dependent like at 0.1

All extracts of MA showed anticancer activity against the breast cancer (MCF-7) cell line. The effect of the MA extract was dose dependant manner. At a conc. of 0.1 µg/ml, aqueous, hydroethanolic and methanolic extract of MA demonstrate 5%, 10% and 24% cell death, at 1 ìg/ml, 11%, 23% and 32% cell death, at 10 µg/ml, 36%, 37% and 49% cell death and at 100 µg/ml concentration, 79%, 77% and 83% cell death, respectively as related to untreated control cells (Tables 6,7 and Figure 5).

Table 6: Anticancer activity of different extracts of MA by MTT assay

Concentrations (µg)	Aqueous extract	Hydroethanolic extract	Methanolic extract	Paclitaxel
0.1	5.37	9.97	23.62	32
1	11.27	22.85	31.67	39
10	35.97	36.65	48.86	45
100	79.01	77.24	83.35	118

Table 7: Anticancer activity of different extracts of MA by MTT assay

Anticancer activity of MA				
Parameter	Methanolic extract	Hydroethanolic extract	Aqueous extract	Paclitaxel
IC_{50} value (µg/ml)	33	50	53	18

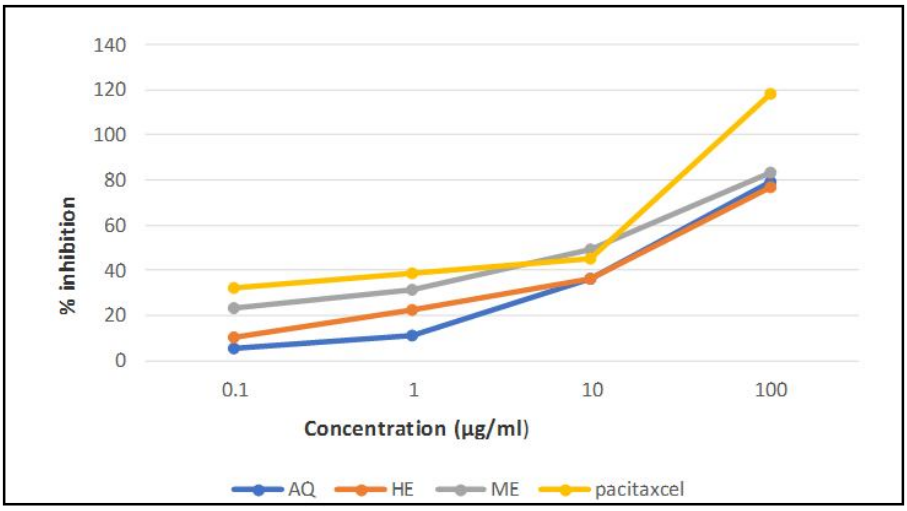


Figure 5: Anticancer activity of MA by MTT assay.

4. Discussion

The primary objective of the study was to examine the anticancer and antioxidant action of the compound Unani herbal formulation MA to corroborate its purported anticancer effects described in Unani literature. Additionally, we conducted phytochemical screening of extracts to identify the active compounds present in this formulation, alongside assessing certain physicochemical standardization parameters. Despite the longstanding use of MA by Unani physicians for cancer treatment, there is a dearth of scientific evidence to support these assertions. This research undertaken, represents an initial step towards validating the Unani tradition by investigating its anticancer properties using modern scientific approaches. Our expectation was that this initial investigation will set the stage for further clinical research into the potential therapeutic applications of this formulation.

Herbal formulation "MA" comprises 14 ingredients, out of that many possessing anticancer and antioxidant properties. One of these ingredients, halela (*Terminalia chebula*), was subjected to testing using the MTT assay on MCF-7 breast cancer cells. The results demonstrated a significant antiproliferative effect and a dose dependent induction of apoptosis. With increasing extract concentration, cell viability gradually decreased, with an IC_{50} of 103.2 µg/ml. The extract exhibited notable inhibition of MCF-7 cell growth, particularly at higher doses, and induced cell cycle arrest at the G1/S phase, impeding cells from entering the DNA replication stage. Additionally, it prompted apoptosis (programmed cell death) in MCF-7 cells. Saccharopine, a phytochemical found in *T. chebula* extract, exhibited robust binding affinity to epidermal growth factor receptors (EGFRs), potentially obstructing their signalling pathways involved in cancer cell growth (Reddy *et al.,* 2023). The fruit's aqueous and alcoholic extracts, rich in phenolic compounds, exhibited notable in vitro free radical scavenging activity at 23 µg/ml, they showed a reduction in DPPH absorption, with an IC_{50} value of 12 ± 2 ìg/ml (Naik *et al.,* 2004). The methanolic extract of Amla (*Emblica officinalis*) fruits displayed the highest free radical scavenging activity as determined by the DPPH assay (ranging from 17.33% to 89.00%) (Middha *et al.,* 2015). According to the study findings, the aqueous extract of *E. officinalis* fruits effectively inhibited the growth of cancer cells in cancer cell lines, including those of breast (MDA-MB-231) cell line, ovarian, lung, cervical, and colorectal cancers. It also demonstrated significant antiinvasive properties against breast cancer cells and exhibited antitumour activity by reducing tumour incidence in mice (Ngamkitidechakul *et al.,* 2010). The red variety of Maweez Munaqqa *(Vitis vinifera),* showed potent cytotoxicity against Capan-2 pancreatic cancer and MDA-MB231 breast cancer cells, especially when combined with seeds (Darwiche *et al.,* 2023). The hydroalcoholic extract of *V. vinifera* showed the maximum antioxidant activity with ABTS, FRAP, DPPH, and ORAC assay (Zeghad *et al.,* 2019). Apoptotic effect of phytochemicals like lycopene, rutin, quercetin, tangeritin, proanthocyanidins and beta-carotene was established by the disturbance in cell viability and induction of apoptosis in cervical cancer Caski cell lines at different doses of these phytochemicals employed in the study as revealed by dose response MTT and Caspase assays. It is noteworthy that most of the naturally occurring agents induce cell apoptosis through caspase-dependent and –independent (Imran *et al.,* 2018). Borage herb (*Borago officinalis*) extracts contain valuable bioactive compounds with antioxidant, antiaging, anti-inflammatory, and protective properties for skin cells (Michalak *et al.,* 2023). The previous study of *Melissa officinalis* reveals that ethanolic extract alone significantly enhanced the mortality of MCF-7 breast cancer cells, but it was less potent than doxorubicin (DOX) in terms of its anticancer activity, with an IC_{50} value of 21.72 µg/ml compared to DOX's IC_{50} value of 0.818 µg/ml, However, when combined with DOX, MOE significantly increased cell mortality compared to DOX treatment alone, reducing the IC_{50} value to 0.425 µg/ml (Hamza *et al.,* 2016). The stem extract of *C. reflexa* demonstrated antioxidant activity as well as cytotoxic activity against lung cancer cell line (A-549) (IC_{50} 436.80 µg/ml) (Elasbali *et al.,* 2023).

Growing evidence suggests that phytochemicals, compounds derived from plants, possess therapeutic properties against various human diseases. Recent studies have shown that phytochemicals like flavonoids, coumarins, *etc*., exhibit promising efficacy in combating cancer with minimal toxicity. These proposed mechanism for their anticancer activity includes programmed cell death, cell migration, and senescence-like cell cycle arrest pathways. The desired effect may be achieved by modulating reactive oxygen species (ROS), the MAPK pathway, the DLC1 pathway, the NF-κB pathway, and glycolytic enzymes (Zheng *et al.,* 2022). It was observed that MA contain alkaloids, flavonoids, glycosides, tannins, phenols and terpenoids *etc*., in good proportion which may be the reason for its antioxidant and cytotoxic properties.

The results of this study reveals that the aqueous extract demonstrated the highest free radical scavenging activity among the three extracts with an IC_{50} value of 31 µg/ml. It means that MA at a concentration of 31 µg/ml, was able to inhibit 50% of the free radicals in the assay. Methanolic extract also exhibited slightly lower activity with an IC_{50} value of 56 µg/ml followed by hydroethanolic extract with an IC_{50} value of 58 µg/ml. In summary, these findings suggest that the aqueous extract is particularly effective in scavenging free radicals, outperforming both the methanolic and hydroethanolic extracts in this specific assay. The study found that different extracts of MA demonstrated varying levels of cytotoxicity on MCF-7 cells, methanolic extract (IC_{50} 33 µg/ml), hydroethanolic extract (IC_{50} 50 µg/ml), and aqueous extract (IC_{50} 53 µg/ml). All extracts of MA exhibited concentration-dependent cytotoxic effects, with the methanolic extract exhibiting the strongest impact on reducing cell viability. The results of this study demonstrates that formulation MA showcased a prominent cytotoxic effect comparable to control drug paclitaxel. It is a strong indicator of its potential efficacy on the MCF-7 cell line.

5. Conclusion

The findings of this research unveiled the presence of bioactive compounds like alkaloids, glycosides, carbohydrates, flavonoids, terpenoids, tannins, saponins, *etc*. The Unani herbal formulation "Matbukh Aftimoon" exhibited antioxidant and cytotoxic properties on MCF-7 cell lines, comparable to standard substances like ascorbic acid and paclitaxel, respectively. This study further corroborates the assertions made by Unani practitioners regarding the potential use of MA in treating breast cancer. The synergistic interaction between the herbs in MA yields a potent anticancer effect, demonstrating promise in combating breast cancer cells and potentially reducing malignancy. Additionally, quality standards have been established, providing a reference for future use. According to the Unani concept, natural drugs resemble the human body, making them generally safe, easily digestible, and metabolizable without producing harmful

secondary metabolites. However, further preclinical and clinical trials are necessary to validate its anticancer potential in humans. This study may serve as a baseline investigation for future research in animals and clinical trials.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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