

Research Article

View Article online



Received 04 May 2025
Revised 23 May 2025
Accepted 07 June 2025
Final review 23 May 2025
Available Online 25 October 2025

Edited by Mohamed Isaqali
Karobari

KEYWORDS:

Antioxidant
Anticancer
Lung cancer
A549
Polyherbal Unani formulation
Total phenolic content
Total flavonoid content
Unani

Natr Resour Human Health 2025; 5 (4): 810–821
<https://doi.org/10.53365/nrfhh/206021>
eISSN: 2583-1194
Copyright © 2025 Visagaa Publishing House

Exploring the Antioxidant and Anticancer Activities of a Polyherbal Unani Formulation on Lung Cancer Cell Line (A549)

Sayed Umera Mujeeb¹, Kuna Lahari², Tirumala Santhoshkumar S³,
Tasleem Ahmad², Javed Inam Siddiqui¹, Younis Iftikhar Munshi⁴,
Mohammad Zakir^{1,A}

¹Ilmul Advia, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.

²BCMB Laboratory, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.

³DSRU, Regional Research Institute of Unani Medicine, Chennai, India.

⁴Incharge Director, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.

ABSTRACT: More and more studies are focusing on natural ingredients as a potential cure for a wide range of diseases. In Unani medicine, several formulations have been described for the management of cancer. An important polyherbal Unani formulation (PUF) has been investigated for its antioxidant and anticancer activities along with total phenolic content (TPC) and total flavonoid content (TFC) in different extracts of PUF. The DPPH and ABTS methods were employed to assess antioxidant activity, while the MTT method was utilised for evaluating anticancer activity. The TPC & TFC were calculated by standard methods. PUF was also subjected to HPTLC fingerprinting, preliminary screening of phytochemicals, and estimation of pesticide residue of the PUF. The extracts were proven to contain alkaloids, flavonoids, saponins, steroids, tannins, glycosides, phenols, and carbohydrates. The highest TPC ($130.5 \pm 8.70 \mu\text{g GAE/mg}$) was found in HE extract while the highest TFC was found in the ME extract of PUF. In DPPH assay, the IC_{50} values of aqueous (AQ), hydroethanolic (HE), and methanolic (ME) were 235, 122, and $188 \mu\text{g/mL}$, respectively, while in ABTS assay, the IC_{50} values of AQ, HE, and ME were 254, 253, and $247 \mu\text{g/mL}$, respectively. In the MTT assay, the IC_{50} value of AQ, HE, and ME was >500 , 100, and $>500 \mu\text{g/mL}$, respectively, confirming that HE extracts showed best activity. The evidence indicates that HE fraction of PUF, exhibits superior antioxidant and anticancer properties, making it a potential candidate for managing cancer and other diseases linked to free radicals.

1. INTRODUCTION

Cancer is defined by the uncontrolled growth of atypical cells that invade neighboring tissues or organs and spread to

other regions of the body (Anonymous, 2025). Breast cancer is the most often diagnosed cancer (11.7%), accounting for 2.3 million new cases, followed by lung cancer (11.4%). Lung cancer is still the biggest cause of cancer-related mortality,

* Corresponding author.

E-mail address: urzakir@rediffmail.com (Mohammad Zakir)

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

accounting for around 1.8 million fatalities or 18% of all cancer deaths (Sung et al., 2021).

Tumor-derived cell lines are the predominant models utilized in cancer research, greatly advancing the comprehension of cancer biology in recent decades. Multiple studies have emphasized genetic disparities between cancer cell lines and tissue specimens (Ertel et al., 2006; Stein et al., 2004). A549 cells represent a commonly utilized human lung carcinoma epithelial cell line, initially isolated from lung tissue affected by adenocarcinoma, and are frequently employed in diverse fields of biomedical research (Domcke et al., 2013).

Hippocrates (Buqrat) is recognized for introducing the term “cancer.” It is derived from the Greek word “cancrum,” which means crab. Hippocrates classifies nonulcer-forming tumors as carcinos and ulcer-forming tumors as carcinomas (Husain et al., 2018). Unani physicians characterize *Saratān* (cancer) as a *Sawdāwī warm* (melancholic inflammation), arising from melancholic humors resulting from the combustion of *Safīrāwī madda* (bile matter) (Sina, 2010). Cancer, also known as *Waram-o-Salāba*, is characterized by significant inflammation, lesions, and branches containing a melancholic humor (*Madda-i-sawdāwī*) (Maseehi et al., 1995).

Cancer is characterised by significant inflammation that is firm and embedded in the tissue, along with tenderness and dryness.. The pressure exerted by the mass exacerbates the discomfort. At first, the growth may be diminutive, resembling a pea, but it has the potential to reach the size of a cantaloupe. The condition is deemed untreatable if it commences with intolerable agony. Nevertheless, the treatment may contribute to the regression of the growth's progression if it occurs without causing significant pain (Hubal, 2005; Sina, 2010).

Contemporary cancer therapies encompass chemotherapy and radiation, both of which entail considerable side effects. Most chemotherapy agents target rapidly proliferating cells, impacting bone marrow, the gastrointestinal system, and hair follicles. These medications may induce myelosuppression, nausea, vomiting, gastrointestinal complications, diminished hematopoiesis, mucositis, alopecia, reproductive diseases such as sterility and infertility, as well as infusion reactions. Moreover, there is an increased susceptibility to infections because of impaired immune function (immunosuppression). (Amjad et al., 2024).

In the Unani medical tradition, various factors influence the onset and progression of cancer, particularly the increased synthesis of black bile (*Sawdā*) and its irregular transformations (Qamri, 2008; Razi, 1991). Galen states that cleansing the body of unhealthy humors requires the use of concoctive for black bile (*Mundij-i-sawdā*) and purgative for black bile (*Mushil-i-sawdā*) (Razi, 1997).

In both in vitro and in vivo investigations, secondary metabolites from plants have exhibited anticancer

properties. Phytochemicals such as Vinblastine, Vincristine, Camptothecin derivatives (Topotecan, Irinotecan), and Homoharringtonine are utilized in cancer therapy (Chandra et al., 2023). Currently, over 3,000 plant species globally have been documented for their anticancer properties. There is a necessity to develop therapeutically validated anticancer drugs from the traditional resources of herbs, minerals, and animals (Solowey et al., 2014). Natural antioxidants reduce the risk of developing conditions, that is, cancer, heart disease, and stroke (Prior et al., 2000). Plant secondary metabolites, including flavonoids and phenolics, exhibit significant free radical scavenging properties (Mathew et al., 2006).

A traditional polyherbal Unani formulation (PUF) containing 11 ingredients was evaluated for its efficacy and safety in a lung cancer cell line. The majority of the ingredients in the PUF have scientifically validated antioxidant and anticancer properties. This formulation is recommended in the management of melancholic diseases (*Sawdāwī amrād*) and serves as a concoctive for black bile (*Mundij-i-sawdā*), a purgative for black bile (*Mushil-i-sawdā*), and a purgative for phlegm (*Mushil-i-balgham*) (Table 1).

This historic Unani concoction, detailed in “Mundij wa mushil” by Majeed Ahmed Narvi, has been selected for examination to establish quality standards, authenticity, antioxidant activity, and anticancer potential by contemporary analytical methodologies (Narvi et al., 1983).

2. MATERIALS AND METHODS

2.1. Crude drugs

The ingredients of the study drug were purchased from a licensed crude drug supplier in Hyderabad. The botanist at

Table 1

Composition of the test formulation.

S. No.	Name	Botanical name	Component	Quantity
1.	Aftimoon	<i>Cuscuta reflexa</i> Roxb.	Branches	3 g
2.	Bisfayej	<i>Polypodium vulgare</i> L.	Root	3 g
3.	Ustukhudus	<i>Lavandula stoechas</i> L.	Whole herb	3 g
4.	Badranjboya	<i>Melissa officinalis</i> L.	Leaves	3 g
5.	Gul-e-Gaozaban	<i>Borago officinalis</i> L.	Flowers	3 g
6.	Berg-e-Gaozaban	<i>Borago officinalis</i> L.	Leaves	6 g
7.	Badiyan	<i>Foeniculum vulgare</i> Mill.	Seeds	6 g
8.	Asl-us-Soos	<i>Glycyrrhiza glabra</i> L.	Root	6 g
9.	Maweez Munaqqa	<i>Vitis vinifera</i> L.	Dried fruit	9 nos.
10.	Anjeer Zard	<i>Ficus carica</i> L.	Fruit	2 nos.
11.	Gul-e-Surkh	<i>Rosa × damascena</i> Herrm.	Dried petals	5 g

the Institute authenticated the crude drugs, and the voucher specimen nos. for *Aftimoon*, *Bisfayej*, *Ustukhudus*, *Badranjboya*, *Berg-e-Gaozaban*, *Gul-e-Gaozaban*, *Badiyan*, *Asl-us-Soos*, *Maweez Munaqqa*, *Anjeer zard*, and *Gul-e-Surkh* were assigned as SMPU/CRI-Hyd-15885, 15886, 15887, 15888, 15889, 15890, 15891, 15892, 15893, 15894, and 15895, respectively.

2.1.1. Preparation of the test drug

The PUF was manufactured in GMP-certified pharmacy licensed under 413/Unani of NRIUMSD, Hyderabad, as per the book titled *Mundij-wa-Mushil*. The individual ingredients were pulverized separately and then mixed to get the final composition. AQ, (1:1) HE, and ME extracts were prepared by mixing 285 g of powdered PUF 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 and extractive values

Organoleptic parameters like texture, color, odor, and taste were evaluated, while extractive values of formulation in water, hydroethanol, and methanol were estimated (QSIMP, 2003; Singh et al., 2023; UPI, 2008).

2.3. Qualitative analysis of phytoconstituents of the extracts

The AQ, HE, and ME extracts of PUF were subjected to test the presence of therapeutically active chemicals like alkaloids, glycosides, fixed oil, etc., by using standard color reaction tests (Venkatesham et al., 2021).

2.4. Sample preparation

Samples of PUF powders (2 g each) were extracted with 20 mL of ethanol using an ultrasonicator for 30 min at a temperature of 45°C, performed in duplicate. The extracts were filtered utilizing Whatman's No. 1 and 41 filter papers prior to being placed into vials for subsequent analysis (Akram et al., 2024).

2.4.1. Chromatographic conditions

The HPTLC chromatogram was executed on a 10 × 10 cm precoated silica gel 60 F₂₅₄ HPTLC plate.

The samples were applied using 8 mm bands with the CAMAG Automatic TLC Sample Applicator IV, which is

equipped with a 100 µl syringe. Twin trough glass chamber with dimensions of 20 cm by 10 cm.

Twin trough glass chamber (dimensions: 20 cm × 10 cm). The optimization of chamber saturation time for the mobile phase was determined to be 20 min at 25°C. The CAMAG TLC Scanner 4 was utilized to detect the densitogram in both absorbance and fluorescence modes at wavelengths of 254 nm, 366 nm, and 520 nm. The plate was applied for derivatization using a Vanillin–Sulfuric acid reagent, followed by scanning at a wavelength of 520 nm. The mobile phase employed for the chromatogram development consists of Toluene, Ethyl acetate, and Formic acid in a ratio of 7.5:2.5:0.01, and the resulting chromatogram was visualized utilizing the CAMAG TLC visualizer. The complete analysis was performed using WinCATS version 1.4.9.2001 operating software. Nitrogen served as the carrier gas for operation under high-pressure conditions. The lamps (Tungsten, Deuterium, and Mercury) were utilized for measuring absorbance values at 254 nm, 366 nm, and 520 nm (Akram et al., 2024).

2.5. Preparation of extract of PUF

The AQ, HE (1:1), and ME extracts of PUF, obtained through cold extraction and subsequently dried using a lyophilizer, were utilized in this study. A stock solution of 10 mg/mL was prepared by dissolving the desiccated extract in dimethyl sulfoxide (DMSO) (Kumar et al., 2023).

2.5.1. DPPH assay

An aliquot of 80 µl of 0.1 mM DPPH in methanol was combined with 20 µl of test extracts at concentrations between 3 and 500 µg/mL in a 96-well micro test plate. The reaction mixture was incubated in darkness, and absorbance was measured at 517 nm after a 30-min interval using a multimode reader (Infinite Pro M 200; Tecan, Austria). Ascorbic acid functioned as the positive control (Rumpf et al., 2023).

The calculation of free radical scavenging activity (% inhibition) was performed using the following formula:

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

2.5.2. ABTS scavenging activity

The ABTS test was conducted as previously outlined, with minor modifications. The formation of ABTS•+ was achieved by preparing a 7 mM solution of ABTS in distilled water, which was then oxidized using 2.45 mM potassium sulfate. The two stock solutions were combined in equal volumes, for 16 hours, to complete the reaction in dark room at normal

temperature. One milliliter of ABTS•+ stock solution was prepared by diluting it with 60 mL of 100% methanol to create the working solution. Twenty microliters of test extracts at specified concentrations (15–500 µg/mL) were combined with 180 µL of ABTS•+ work reagent in a 96-micro well plate and incubated for 2 h at room temperature in the dark. Gallic acid was measured at 734 nm using a Tecan Multimode reader (Re et al., 1999).

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

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

2.6. Cytotoxicity Activity

A549 cells were cultured in a T-25 flask with a medium consisting of DMEM, 10% heat-inactivated FBS, 1% 200 mM L-glutamine, and 0.5% antibiotic anti-mycotic solution. The cells were cultured in a CO₂ incubator with 95% air, 100% relative humidity, and 5% CO₂ at 37°C. Cultures were subcultured every sixth day, and the media was refreshed three times weekly. Viable cell counts were assessed through the Trypan Blue exclusion method utilizing Hemacytometers (McCauley et al., 2013).

2.6.1. MTT Assay

The cytotoxicity was evaluated by the MTT assay, a colorimetric method. Cells were detached at approximately 90% confluency using 0.05% trypsin/EDTA. The cells were subsequently resuspended and inoculated into a 96-well plate at a volume of 200 µL per well. Equal cell quantities per well were subjected to varying doses of the test chemical on a logarithmic scale. Following 24-h incubation, the cells were rinsed with PBS, after which MTT (0.5 mg/mL in PBS) was added to each well and incubated at 37°C for 3 h. Formazan crystals were solubilized by adding DMSO (100 µL/well), and absorbance was measured at a wavelength of 570 nm using a Microplate Reader. The untreated cells served as the control group, exhibiting 100% viability. Paclitaxel was employed as the standard treatment. All concentrations were replicated in pairs, and results were derived from three measurements. The IC₅₀ value will indicate the effectiveness of cell growth inhibition for each extract (McCauley et al., 2013).

The percentage of inhibition of the extract against the cell line was calculated using the formula provided below:

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

$$\% \text{ Cell inhibition} = 100 - \% \text{ Cell viability}$$

2.7. Quantitative analysis of total phenols (TPC)

The TPC was determined using the Folin–Ciocalteu reagent as described. Briefly, 30 µL of extracts were combined with 150 µL of 1:10 Folin–Ciocalteu reagent. After 5 mins of incubation, add 120 µL of 7% Na₂CO₃ and mix thoroughly. After 2 h of incubation in the dark, the absorbance at 750 nm was measured using a multimode reader (Infinite Pro M 200 Tecan, Austria). Gallic acid was utilized as the standard reference, and total phenols were reported in gallic acid equivalents (GAE) (Farasat et al., 2013).

2.8. Quantitative analysis of total flavonoid (TFC)

The amount of TFC was estimated using the quercetin standard curve and represented as quercetin equivalent (QE) using the aluminum chloride colorimetric test. To put it briefly, 10 µL of 10% aluminum chloride solution, 150 µL of 95% ethanol, and 10 µL of 1 M sodium acetate were added to a micro-well plate together with 50 µL of extracts (1000 µg/mL) and/or a standard solution of quercetin (50, 100, 150, 200, and 250 µg/mL) in 80% ethanol. After thoroughly mixing all the ingredients, they were incubated in the dark for 40 min. Using a multimode reader (Infinite pro M 200 Tecan, Austria), the absorbance was measured at 415 nm (Infinite pro M 200 Tecan, Austria) (Eddahhaoui et al., 2022).

2.7. Statistical analysis

The statistical calculation was performed using Graph Pad Prism 5.0, and ANOVA was conducted with the assistance of Open Epi software. The values of $p \leq 0.05$ were regarded as statistically significant.

3. RESULTS

3.1. Organoleptic Evaluation and extractive values

Appearance: Coarse powder; Color: Light brown; Odor: Fragrance; and Taste: Slightly sweet. The mean concentrations of aqueous, hydroethanolic, and methanolic extracts (% w/w) were determined to be 4.78, 31.43, and 21.68, respectively.

3.3. Qualitative phytochemical analysis

The findings regarding the color reaction indicative of secondary metabolites are illustrated in Table 2. It has been noted

Table 2

Phyto-constituents of polyherbal Unani formulation.

S. No.	Constituents	Results		
		Hydroethanolic extract	Methanolic extract	Aqueous extract
1.	Alkaloids	+++	+	++
2.	Glycosides	—	—	—
3.	Carbohydrate	+	—	+++
4.	Flavonoids	++	++	+++
5.	Saponins	++	+	—
6.	Steroids	+++	+++	+++
7.	Tannin	++	++	+++
8.	Proteins	—	—	++
9.	Phenols	—	++	+++
10.	Resins	—	—	—

that all extracts exhibited substantial levels of chemical constituents. The findings indicate the existence of therapeutically significant secondary metabolites, including alkaloids, flavonoids, saponins, tannins, proteins, and phenols, among others.

3.4. HPTLC of PUF

The HPTLC analysis demonstrated the existence of multiple phytochemical constituents in the ethanol extract of PUF, identified under two separate ultraviolet (UV) wavelengths. Under UV light at 254 nm, five significant major peaks were observed at R_f values of 0.01 (48.68%), 0.08 (8.15%), 0.18 (27.81%), 0.88 (8.23%), and 0.97 (7.13%). At 366 nm, seven major peaks were identified with R_f values of 0.01 (23.24%), 0.08 (62.88%), 0.13 (1.32%), 0.16 (1.04%), 0.21 (5.61%), 0.34 (4.16%), and 0.82 (1.16%). In addition, seven major peaks were observed with R_f values of 0.01 (24.28%), 0.14 (2.96%), 0.16 (6.53%), 0.35 (13.82%), 0.45 (7.84%), 0.65 (36.30%), and 0.98 (8.27%), as presented in Table 3. The chromatographic profiles demonstrate a unique chemical fingerprint of the ethanol extract of PUF, indicative of the solvent system used. The fingerprinting results, along with the 3D densitogram and the associated R_f values, as shown in Figure 1, validate the existence of active phytoconstituents in the extract. The extensive data gathered act as a valuable resource for identifying and characterizing phytoconstituents in PUF, offering a dependable reference for upcoming research (Christopher et al., 2024).

3.5. DPPH radical scavenging activity

All extracts of PUF showed dose-dependent antioxidant activity, increasing with higher concentration. Result showed

highest activity in HE extracts with IC₅₀ value 108 µg/mL, followed by ME and AQ extract when equated with ascorbic acid (Table 4 and Figure 2).

3.6. ABTS radical scavenging activity

All extracts of PUF showed dose-dependent antioxidant activity, increasing with higher concentration. Result showed almost similar activity in all extracts of the PUF (Table 5 and Figure 3).

3.7. MTT assay

The cytotoxicity activity was evaluated at different concentrations extending 7.5, 31.25, 125, and 500 µg/mL, and subsequently IC₅₀ was calculated. The highest activity was demonstrated by HE extracts with exhibited IC₅₀ value of 100 µg/mL. The PUF has demonstrated the dose-dependent activity in all extracts (Table 6 and Figure 4).

3.8. TPC

The TPC was measured using gallic acid (Figure 5) and expressed in milligrams of GAE. The extracts demonstrated a notable concentration of phenolic compounds, varying from 64.32±1.33 to 130.5±8.7 mg of GAE/g of extract. The methanolic extract exhibited the highest total phenolic concentration (Table 7).

3.9. TFC

Total TFC was estimated by Quercetin (Figure 6) and expressed as milligrams of QE. All the extracts contained a considerable amount of TFCs from 12±0.9 to 64±7.7 mg of QE/g of extract. Methanolic extract exhibited the highest total TFC (Table 7).

3.10. Pesticidal residue screening of PUF

The analysis of pesticide residues for PUF was conducted using the validated test method R-44 at Bureau Veritas India Testing Services, Private Limited, Sanath Nagar, Hyderabad, Telangana (Table 8). The testing was performed following the test method BVTs/FOOD/INS/SOP-040 for various pesticide residues, and the results are detailed in Table 8.

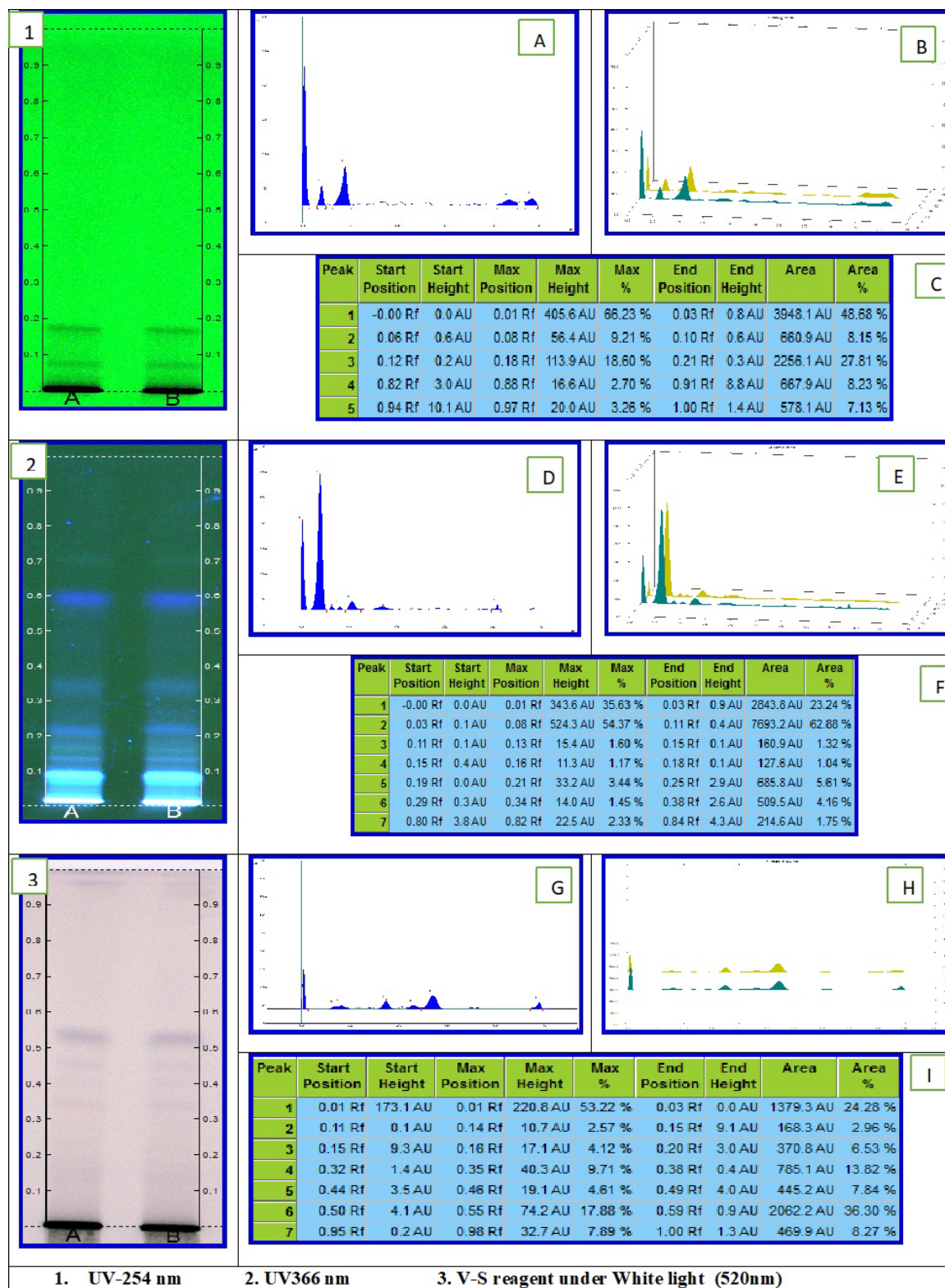


Figure 1. Rf values of HPTLC of alcoholic extract of polyherbal Unani formulation. (A) HPTLC finger print of PUF in alcohol extract at 254 nm, (B) 3D densitometry chromatogram of PUF at 254 nm, (C) peak table of PUF at 254 nm, (D) HPTLC finger print of PUF in alcohol extract at 366 nm, (E) 3D densitometry chromatogram of PUF at 366 nm, (F) peak table of PUF at 366 nm, (G) V-S reagent finger print of PUF in alcohol extract at 520 nm, (H) 3D densitometry chromatogram of PUF at 520 nm, and (I) peak table of PUF at 520 nm.

Table 3

Ethanollic extract of polyherbal Unani formulation observed phyto-constituents in various ultraviolet wavelengths.

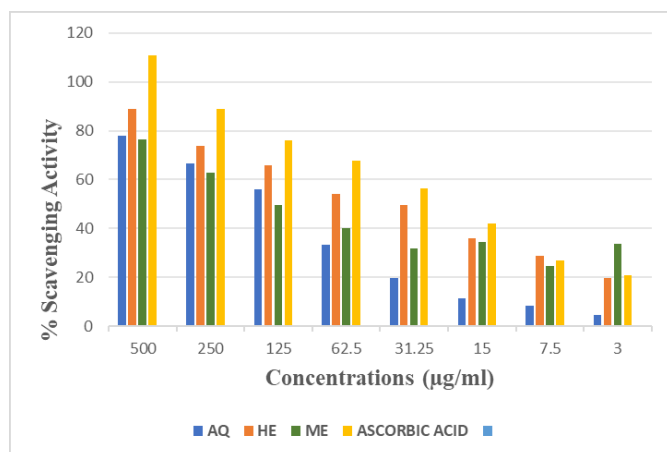
Solvent system	UV—254 nm		UV—366 nm		White Light UV—520 nm	
	Rf	Color	Rf	Color	Rf	Color
Toluene: ethyl	0.01	LG	0.01	FB	0.01	LGR
acetate: formic acid	0.08	G	0.08	B	0.14	LGR
7.5: 2.5:0.01	0.18	DG	0.13	LR	0.16	LGR
	0.88	G	0.16	B	0.35	LR
	0.97	G	0.21	B	0.45	LB
			0.34	LB	0.65	DV
			0.82	LG	0.98	LV

G = green; LG = light green, DG = dark green, FB = Fluorescent blue, LR = light red; B = blue, LB = light blue, LGR= light gray, DV = Dark violet, LV = Light violet

Table 4

Antioxidant activity of polyherbal Unani formulation by DPPH assay.

IC ₅₀ values of different extracts of polyherbal Unani formulation				
Extracts	Aqueous	Methanolic	Hydroethanolic	Ascorbic
IC ₅₀ value (µg/mL)	221	180	108	47

**Figure 2.** Antioxidant activity of polyherbal Unani formulation by DPPH assay.

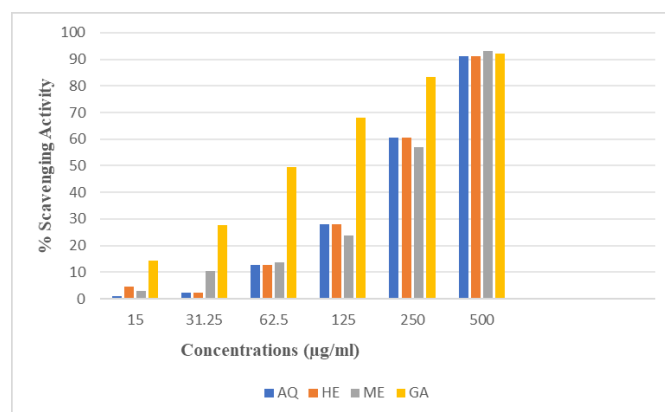
4. DISCUSSION

The main aim of this study was to investigate the anticancer and antioxidant properties of PUF in order to validate its claimed anticancer effects as outlined in Unani literature. In addition, we conducted phytochemical screening of extracts to identify the active compounds present in this formulation, alongside assessing certain physicochemical standardization

Table 5

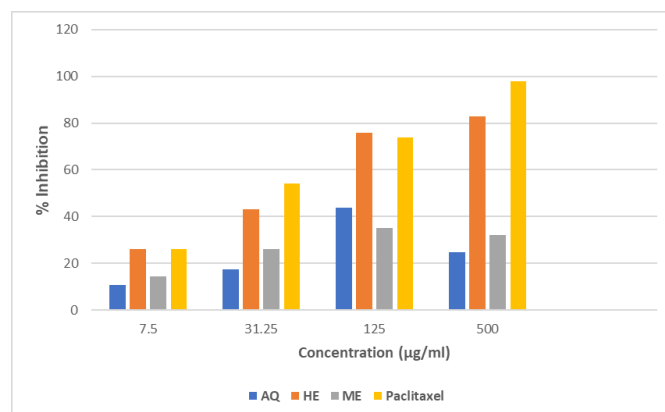
Antioxidant activity of polyherbal Unani formulation by ABTS assay.

IC ₅₀ values of different extracts of polyherbal Unani formulation				
Extracts	Aqueous	Methanolic	Hydroethanolic	Galic acid
IC ₅₀ value (µg/mL)	254	247	253	4.33

**Figure 3.** Antioxidant activity of polyherbal Unani formulation by ABTS assay.**Table 6**

Anticancer activity of polyherbal Unani formulation by MTT assay.

IC ₅₀ values of different extracts of polyherbal Unani formulation				
Extracts	Methanolic	Hydroethanolic	Aqueous	Paclitaxel
IC ₅₀ value (µg/mL)	>500	100	>500	54

**Figure 4.** MTT Assay of different extracts of polyherbal Unani formulation.

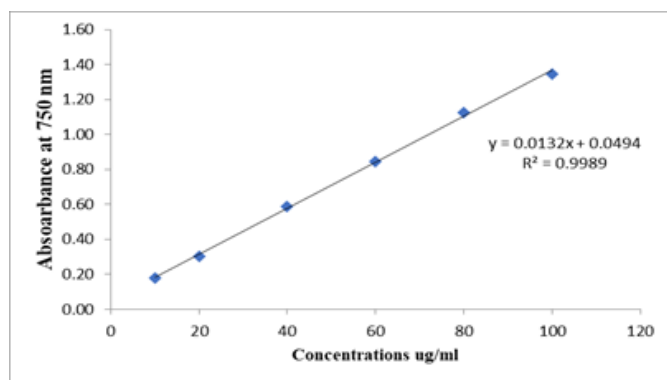


Figure 5. Standard curve for gallic acid.

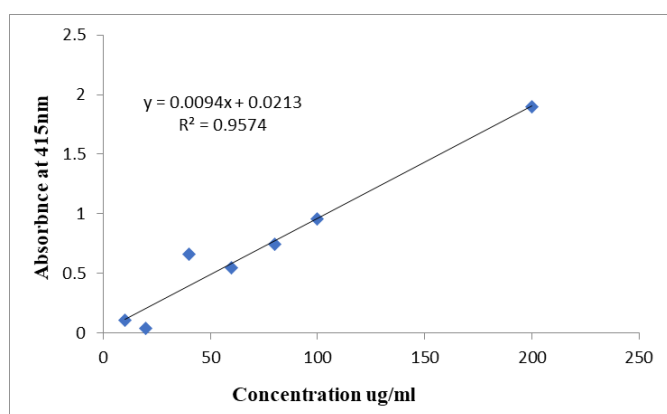


Figure 6. Standard curve for quercetin.

parameters. In spite of the longstanding use of PUF by Unani physicians for the treatment of cancer, 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.

Unani formulation “PUF” comprises 11 ingredients, out of that many possess anticancer and antioxidant properties as per Unani literature (Narvi et al., 1983). Recent studies showed that most of the ingredients have shown the anticancer and antioxidant properties (Table 9).

Growing evidence suggests that phytochemicals, derived from plants, possess therapeutic properties against various human diseases. Recent studies have shown that phytochemicals like flavonoids, phenols, coumarins, etc., exhibit promising efficacy in combating cancer with minimal toxicity. The proposed mechanism for their anticancer activity includes programmed cell death, cell migration, and senescence-like

Table 7

Total phenols’ and flavonoids’ content of the aqueous, hydroethanolic, and methanolic extracts of polyherbal Unani formulation.

Extracts	Total phenol ($\mu\text{g GAE/mg}$)	Total flavonoids ($\mu\text{g QE/mg}$)
Aqueous (AQ)	64.32 $\pm$ 1.33	12 $\pm$ 0.9
Hydroethanol (HE)	101.7 $\pm$ 1.37	55 $\pm$ 2.6
Methanol (ME)	130.5 $\pm$ 8.7	64 $\pm$ 7.7

cell cycle arrest pathways. The desired effect may be achieved by modulating reactive oxygen species (ROS), the MAPK pathway, the DLC1 pathway, the NF- κ B pathway, and glycolytic enzymes (Zheng et al., 2022). It was observed that PUF contains alkaloids, phenols, flavonoids, saponins, tannins, proteins, etc., in good proportion, which may be the reason for its antioxidant and cytotoxic properties. The findings of this investigation indicate that the HE extract exhibited the most significant free radical scavenging activity among the three extracts in DPPH as well as ABTS assay.

The research revealed that various extracts of PUF exhibited differing degrees of cytotoxicity on A549 cells, with the HE extract showing the highest level. All extracts of PUF had cytotoxic effects that were dependent on concentration. The maximum TPC was seen in the HE extract, but the highest TFC was recorded in the ME extract of PUF. The substantial concentration of TPC and TFC may corroborate the antioxidant and anticancer efficacy of the PUF. The findings of this investigation indicate that PUF exhibited a significant cytotoxic impact comparable to the control medication paclitaxel. This strongly indicates its potential efficacy on the A549 cell line.

5. CONCLUSION

The PUF contains good amount of TPC and TFC along with alkaloids, saponins, tannins, proteins, etc. The presence of secondary metabolites justifies the antioxidant and cytotoxic effects of PUF against the A549 cell line. The best free radical scavenging and anticancer activity was demonstrated by hydroethanolic extracts of PUF. Emphasizing the benefits of the Unani system, particularly its reliance on natural and easily metabolized substances, could improve comprehension and possibly foster greater acceptance of traditional medicinal practices. The findings reinforce the Unani methodology of employing natural drugs in their unaltered state. Because of the preliminary nature of the study, additional research, particularly involving animal models and subsequent clinical trials, is recommended.

Table 8

Pesticide residue analysis for polyherbal Unani formulation.

S. No.	Test Parameters in mg/kg	Test Result	LOQ	S. No.	Test Parameters in mg/kg	Test Result	LOQ
1	Alachlor	BLQ	0.01	26	Endosulfan sulfate	BLQ	0.01
2	Aldrin, dieldrin	BLQ	0.01	27	Endrin	BLQ	0.01
3	Dieldrin	BLQ	0.01	28	Ethion	BLQ	0.01
4	Azinophos methyl	BLQ	0.01	29	Fenitrothion	BLQ	0.01
5	Bromopropylate	BLQ	0.01	30	Fenvalerate	BLQ	0.01
6	Chlordane cis	BLQ	0.01	31	Heptachlor	BLQ	0.01
7	Chlordane trans	BLQ	0.01	32	Heptachlor epoxide	BLQ	0.01
8	Chlorfenvinphos	BLQ	0.01	33	Hexa-chlorobenzene	BLQ	0.01
9	Chlorpyrifos	BLQ	0.01	34	Alpha-HCH	BLQ	0.01
10	Chlorpyrifos methyl	BLQ	0.01	35	Beta-HCH	BLQ	0.01
11	Cypermethrin I	BLQ	0.01	36	Delta-HCH	BLQ	0.01
12	Cypermethrin II	BLQ	0.01	37	Methidathion	BLQ	0.01
13	Cypermethrin III	BLQ	0.01	38	Parathion	BLQ	0.01
14	Cypermethrin IV	BLQ	0.01	39	Methyl parathion	BLQ	0.01
15	O,P-DDT	BLQ	0.01	40	Permethrin I	BLQ	0.01
16	P,P-DDT	BLQ	0.01	41	Permethrin II	BLQ	0.01
17	O,P-DDE	BLQ	0.01	42	Phosalone	BLQ	0.01
18	P,P-DDE	BLQ	0.01	43	Piperonyl butoxide	BLQ	0.01
19	P,P-DDD	BLQ	0.01	44	Pirimiphos-methyl	BLQ	0.01
20	Deltamethrin	BLQ	0.01	45	Pyrethrins	BLQ	0.01
21	Diazinon	BLQ	0.01	46	Quintozone	BLQ	0.01
22	Dichlorvos	BLQ	0.01	47	Pentachloroaniline	BLQ	0.01
23	Dithiocarbamates	BLQ	0.01	48	Pentachlorophenyl methyl sulfide	BLQ	0.01
24	Endosulfan alpha	BLQ	0.01	49	Malathion (malathon and malaaxon)	BLQ	0.01
25	Endosulfan beta	BLQ	0.01	50	Lindane (gamma isomers of hexachlorocyclohexane)	BLQ	0.01

*BLQ – below the limit of quantification; LOQ – limit of quantification.

Table 9

Therapeutic action, uses, and anticancer and antioxidant activities of the ingredients of polyherbal Unani formulation.

Drugs	Therapeutic actions and uses as per Unani	Antioxidant & anticancer activity studies
<i>Cuscuta reflexa</i>	Anti-inflammatory, tonic, concoctive of black bile and purgative of black bile. Cancer and melancholia (Ghani, 2011).	Anticancer and antioxidant activity (Elasbali et al., 2024; Singh et al., 2022).
<i>Polypodium vulgare</i>	Anticonvulsant, expectorant, and purgative of black bile and phlegm. Asthma and diseases of black bile and phlegm (Ghani, 2011).	Anticancer and antioxidant activity (Batur et al., 2020; Tabeshpour et al., 2023).
<i>Lavandula stoechas</i>	Tonic and purgative of black bile and phlegm. Schizophrenia and coryza (Ghani, 2011).	Anticancer and antioxidant activity (El Hachlafi et al., 2023; Syaj et al., 2025).
<i>Melissa officinalis</i>	Concoctive of black bile and sedative. Melancholic and phlegmatic disorders (Ghani, 2011).	Anticancer and antioxidant activity (Draginic et al., 2022; Kuo et al., 2021).
<i>Borago officinalis</i>	Purgative to black bile and tonic. Cough and pharyngitis (Ghani, 2011).	Anticancer and antioxidant activity (Michalak et al., 2023; Singh et al., 2017).
<i>Foeniculum vulgare</i>	Analgesic, concoctive of phlegm and black bile, and anti-inflammatory. Bronchial asthma and cough (Ghani, 2011).	Anticancer and antioxidant activity (Di Napoli et al., 2022; Ghasemian et al., 2020).
<i>Glycyrrhiza glabra</i>	Concoctive of phlegm, cleanses respiratory passage, concoctive of viscous humor. Bronchitis and diseases of phlegm and black bile (Ghani, 2011).	Anticancer and antioxidant activity (Caroline et al., 2023; Zhou et al., 2019).
<i>Vitis vinifera</i>	Tonic, concoctive of viscous humor, concoctive for phlegm and black bile. General debility, melancholic diseases, and cancer (Ghani, 2011).	Anticancer and antioxidant activity (Lantzouraki et al., 2020; Tsantila et al., 2024).
<i>Ficus carica</i>	Tonic, anti-inflammatory, concoctive, and demulcent for lungs. Schizophrenia (Ghani, 2011).	Anticancer and antioxidant activity (Ayoub et al., 2019; Purnamasari et al., 2019).
<i>Rosa × damascena</i>	Analgesic, purgative of phlegm and black bile, and anti-inflammatory. Syncope and pharyngitis (Ghani, 2011).	Anticancer and antioxidant activity (Shokrzadeh et al., 2017; Zahedi-Amiri et al., 2019).

ACKNOWLEDGMENT

The authors thank the Director General of CCRUM, New Delhi, for encouraging and providing facilities for this research.

AUTHOR CONTRIBUTIONS

Mohammad Zakir was responsible for conceptualization, methodology, supervision, writing—original draft, and reviewing and editing; Sayed Umera Mujeeb did the investigation, writing—reviewing and editing; Kuna Lahari did formal analysis; Tirumala Santhoshkumar S did investigation for quality control; Tasleem Ahmed did the Investigation; Javed Inam Siddiqui was involved in writing, reviewing, and editing; and Younis Iftikhar Munshi took care of project administration and resources.

CONFLICTS OF INTEREST

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

FUNDING

None.

ORCID

Sayed Umera Mujeeb	0009-0007-1251-3881
Kuna Lahari	0009-0003-3214-6311
Tirumala Santhoshkumar S	0000-0003-1259-8671
Tasleem Ahmad	0000-0002-9568-4753
Javed Inam Siddiqui	0000-0002-7275-6208
Younis Iftikhar Munshi	0000-0001-7784-0196
Mohammad Zakir	0000-0003-3003-2292

REFERENCES

- Akram, S.A.W., Arokiarajan, M.S., Christopher, J.J., Jameel, M., Saquib, M., Saripally, T.S.K., Anwar, N., Asif, M., Ahmed K.K. 2024. Antimicrobial and antioxidant study of combined essential oils of *Anethum Sowa* Kurz., and *Trachyspermum ammi* (L.) along with quality determination, comparative histo-anatomical features, GC-MS and HPTLC chemometrics. Scientific Reports. 14, 27010. <https://doi.org/10.1038/s41598-024-75773-8>.
- Amjad, M.T., Chidharla, A., Kasi, A. 2024. Cancer chemotherapy. 2023 Feb 27. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Bookshelf ID:NBK564367
- Anonymous. National Cancer Institute. 2025. What is cancer? Available from: <https://www.cancer.gov/about-cancer/understanding/what-is-cancer>. (accessed on 29.04.2025)
- Ayoub, L., Hassan, F., Hamid, S., Abdelhamid, Z., Souad, A. 2019. Phytochemical screening, antioxidant activity and inhibitory potential of *Ficus carica* and *Olea europaea* leaves. Bioinformation 15(3), 226–232. <https://doi.org/10.6026/97320630015226>.
- Batur, S., Ayla, S., Sakul, A.A., Okur, M.E., Karadag, A.E., Daylan, B., Ozdemir, E.M., Kepil, N., Gunal, M.Y. 2020. An alternative approach wound healing field with polypodium vulgare. Medeniyet Medical Journal 35(4), 315–323. <https://doi.org/10.5222/MMJ.2020.89983>
- Caroline, M.L., Muthukumar, R.S., AH,H.P., N.N. 2023. Anticancer effect of plectranthus amboinicus and glycyrrhiza glabra on oral cancer cell line: An invitro experimental study. Asian Pacific Journal of Cancer Prevention 24(3), 881–887. <https://doi.org/10.31557/APJCP.2023.24.3.881>
- Chandra, S., Gahlot, M., Choudhary, A.N., Palai, S., de Almeida, R.S., de Vasconcelos, J.E.L., dos Santos, F.A.V., de Farias, P.A.M., Coutinho, H.D.M., 2023. Scientific evidences of anticancer potential of medicinal plants. Food Chemistry Advances 2, 100239. <https://doi.org/10.1016/j.focha.2023.100239>
- Christopher, J.J., Akram, S.A.W., Arokiarajan, M.S., Jameel, M., Saripalli, T.S.K., Ahmed, V.M., Anwar, N., Ahmad, K.K. 2024. The qualitative and histoanatomical validation of *Daucus carota* L., fruit along with in vitro cytotoxic, antioxidants and antimicrobial analysis. Food and Humanity 3, 100326. <https://doi.org/10.1016/j.foohum.2024.100326>.
- Di Napoli, M., Castagliuolo, G., Badalamenti, N., Maresca, V., Basile, A., Bruno, M., Varcamonti, M., Zanfardino, A. 2022. Antimicrobial, antibiofilm, and antioxidant properties of essential oil of *foeniculum vulgare* mill. leaves. Plants 11(24), 3573. . <https://doi.org/10.3390/plants11243573>.
- Domcke, S., Sinha, R., Levine, D.A., Sander, C., Schultz, N. 2013. Evaluating cell lines as tumour models by comparison of genomic profiles. Nature Communications 4(1), 2126. <https://doi.org/10.1038/ncomms3126>
- Draginic, N., Andjic, M., Jeremic, J., Zivkovic, V., Kocovic, A., Tomovic, M., Bozin, B., Kladar, N., Bolevich, S., Jakovljevic, V., Milosavljevic, I. 2022. Anti-inflammatory and antioxidant effects of *Melissa officinalis* extracts: A comparative study. Iranian Journal of Pharmaceutical Research 21(1), e126561. <https://doi.org/10.5812/ijpr-126561>.
- Eddahhaoui, F.Z., Boudalia, M., Harhar, H., Chahboun, N., Tabyaoui, M., Guenbour, A., Zarrouk, A., Bellaouchou, A. 2022. Effect of the extraction technique on the bioactive compounds and the antioxidant capacity of the *chamaerops humilis* L. fruit (pulp and seeds). Chemical Data Collections 40, 100882. <https://doi.org/10.1016/j.cdc.2022.100882>.
- El Hachlafi, N., Benkhaira, N., Al-Mijalli, S.H., Mrabti, H.N., Abdnim, R., Abdallah, E.M., Jeddi, M., Bnouham, M., Lee, L.H., Ardianto, C., Ming, L.C. 2023. Phytochemical analysis and evaluation of antimicrobial, antioxidant, and antidiabetic activities of essential oils from Moroccan medicinal plants: *Mentha suaveolens*, *Lavandula stoechas*, and *Ammi visnaga*. Biomedicine

- & Pharmacotherapy 164, 114937. <https://doi.org/10.1016/j.biopha.2023.114937>.
- Elasbali, A.M., Al-Soud, W.A., Mousa Elayyan, A.E., Alhassan, H.H., Danciu, C., Elfaki, E.M., Alharethi, S.H., Alharbi, B., Alanazi, H.H., Mohtadi, M.E., Patel, M. 2024. Antioxidative and ROS-dependent apoptotic effects of *Cuscuta reflexa* Roxb. stem against human lung cancer: Network pharmacology and in vitro experimental validation. *Journal of Biomolecular Structure and Dynamics* 42(21), 11651–11676. <https://doi.org/10.1080/07391102.2023.2263889>.
- Ertel, A., Verghese, A., Byers, S.W., Ochs, M., Tozeren, A. 2006. Pathway-specific differences between tumor cell lines and normal and tumor tissue cells. *Molecular Cancer* 555–13. <https://doi.org/10.1186/1476-4598-5-55>
- Farasat, M., Khavari-Nejad, R.A., Nabavi, S.M.B., Namjooyan, F. 2013. Antioxidant properties of two edible green seaweeds from northern coasts of the Persian Gulf. *Jundishapur Journal of Natural Pharmaceutical Products* 8(1), 47.
- Ghani, H.N. Khazain-al-advia. 2011. CCRUM, Ministry of H & FW, Govt. of India, New Delhi pp. 226–227; 242–243; 279–280; 317–319; 325; 370–371; 390–391; 1116–117; 1133–1135; 1137; 1260–1261.
- Ghasemian, A., Al-Marzoqi, A.H., Mostafavi, S.K.S., Alghanimi, Y.K., Teimouri, M. 2020. Chemical composition and antimicrobial and cytotoxic activities of *foeniculum vulgare* mill essential oils. *Journal of gastrointestinal cancer* 51, 260–266. <https://doi.org/10.1007/s12029-019-00241-w>.
- Hubal, I. 2005. *Kitabul Mukhtarat Fil Tibb*, (Urdu translation) Vol–IV, CCRUM, Ministry of Health and Family Welfare, Govt. of India, New Delhi, pp.182–183.
- Husain, M.K., Khalid, M., Penchala Pratap, G., Kazmi, M.H. 2017. Relevance of traditional unani (Greco-Arab) system of medicine in cancer: An update. In: Akhtar, M., Swamy, M. (eds) *Anticancer Plants: Clinical Trials and Nanotechnology*. Springer, Singapore. pp. 273–302. https://doi.org/10.1007/978-981-10-8216-0_10
- Kumar, A.P.N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T.K.S., Oz, F. 2023. Major phytochemicals: recent advances in health benefits and extraction method. *Molecules* 28(2), 887. <https://doi.org/10.3390/molecules28020887>
- Kuo, T.T., Chang, H.Y., Chen, T.Y., Liu, B.C., Chen, H.Y., Hsiung, Y.C., Hsia, S.M., Chang, C.J., Huang, T.C. 2020. *Melissa officinalis* extract induces apoptosis and inhibits migration in human colorectal cancer cells. *Acs Omega* 5(49), 31792–31800. <https://doi.org/10.1021/acsomega.1c00428>.
- Lantzouraki, D.Z., Tsiaka, T., Soteriou, N., Asimomiti, G., Spanidi, E., Natskoulis, P., Gardikis, K., Sinanoglou, V.J., Zoumpoulakis, P. 2020. Antioxidant profiles of *vitis vinifera* L. and *salvia triloba* L. leaves using high-energy extraction methodologies. *Journal of AOAC International* 103(2), 413–421. <https://doi.org/10.5740/jaoacint.19-0261>.
- Maseehi, A.A.I.Q. 1995. *Kitabul Umda fil Jarahat*, (Urdu translation), Vol–I & II, CCRUM, Ministry of Health and Family Welfare, Govt. of India, New Delhi, pp.169–175.
- Mathew, S. and Abraham, T.E. 2006. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food and Chemical Toxicology* 44(2), 198–206. <https://doi.org/10.1016/j.fct.2005.06.013>
- McCauley J, Zivanovic A, Skropeta D. 2013. Bioassays for anticancer activities. In: Roessner, U., Dias, D. (eds). *Metabolomics Tools for Natural Product Discovery*. *Methods in Molecular Biology*, vol 1055. Humana Press, Totowa, NJ. 191–205. https://doi.org/10.1007/978-1-62703-577-4_14
- Michalak, M., Zagórska-Dziok, M., Klimek-Szczykutowicz, M., Szopa, A. 2023. Phenolic profile and comparison of the antioxidant, anti-ageing, anti-inflammatory, and protective activities of *Borago officinalis* extracts on skin cells. *Molecules* 28(2), 868. <https://doi.org/10.3390/molecules28020868>.
- Narvi, M.A. 1983. *Mundij wa Mushil*, Asrar Karimi Press, Allahabad, pp. 53–54.
- Prior, R.L. and Cao, G. 2000. Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. *HortScience* 35(4), 588–592. <https://doi.org/10.21273/HORTSCI.35.4.588>
- Purnamasari, R., Winarni, D., Permanasari, A.A., Agustina, E., Hayaza, S., Darmanto, W. 2019. Anticancer activity of methanol extract of *Ficus carica* leaves and fruits against proliferation, apoptosis, and necrosis in Huh7it cells. *Cancer Informatics* 18, 1176935119842576. <https://doi.org/10.1177/1176935119842576>.
- Qamri, M.H. 2008. *Ghina Muna* (Urdu translation), CCRUM, Ministry of Health and Family Welfare, Govt. of India, New Delhi, pp. 508–510.
- QSIMP. 2003. Quality standards of Indian medicinal plants, Indian council of medical research, Vol–I, New Delhi, pp. 205–111.
- Razi, A.B.M.Z. 1991. *Kitab al-Mansuri* (Urdu translation), CCRUM, Ministry of Health and Family Welfare, Govt. of India, New Delhi, pp. 266–267.
- Razi, A.B.M.Z. 1997. *Al Hawi Fil Tib* (Urdu translation), Vol. 12, CCRUM, Ministry of Health and Family Welfare, Govt. of India, New Delhi, pp. 9–24.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26(9–10), 1231–1237. [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3).
- Rumpf, J., Burger, R., Schulze, M. 2023. Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. *International Journal of Biological Macromolecules* 233, 123470. <https://doi.org/10.1016/j.ijbiomac.2023.123470>.
- Shokrzadeh, M., Habibi, E., Modanloo, M. 2017. Cytotoxic and genotoxic studies of essential oil from *Rosa damascene* Mill., Kashan, Iran. *Medicinski Glasnik* 14(2), 152–157. <https://doi.org/10.17392/901-17>.
- Sina, I. (2010). *Al Qanoon Fit Tib*, Vol-4, Idara Kitabus Shifa, New Delhi, pp. 1278–1280.
- Singh, A., Singh, V., Ananthan, R., Kumar, B.D. 2022. Evaluation of immunomodulatory and antioxidant properties of traditional Kwath, conventional extracts of plants *Cocculus hirsutus* and *Cuscuta reflexa*—in vitro & ex vivo studies. *Journal of Ayurveda and Integrative Medicine*. 13(1), 100537. <https://doi.org/10.1016/j.jaim.2021.100537>.
- Singh, H., Du, J., Yi, T.H. 2017. Green and rapid synthesis of silver nanoparticles using *Borago officinalis* leaf extract: Anticancer and antibacterial activities. *Artificial Cells, Nanomedicine, and Biotechnology* 45(7), 1310–1316. <https://doi.org/10.1080/21691401.2016.1228663>.
- Singh, R.P., Goswami, S., Pathak, S. 2023. HPTLC fingerprinting of quercetin and assessment of in vitro and in vivo antihyperglycemic effect of the leaves of *Tagetes erecta* L. *Annals of Phytomedicine* 12(2), 562–571. <http://dx.doi.org/10.54085/ap.2023.12.2.66>.

- Solowey, E., Lichtenstein, M., Sallon, S., Paavilainen, H., Solowey, E., Lorberboum-Galski, H. 2014. Evaluating medicinal plants for anticancer activity. *The Scientific World Journal* 2014(1), 721402. <https://doi.org/10.1155/2014/721402>
- Stein, W.D., Litman, T., Fojo, T., Bates, S.E. 2004. A serial analysis of gene expression (SAGE) database analysis of chemosensitivity: Comparing solid tumors with cell lines and comparing solid tumors from different tissue origins. *Cancer Research* 64(8), 2805–2816. <https://doi.org/10.1158/0008-5472.CAN-03-3383>
- Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F. 2021. 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. <https://doi.org/10.3322/caac.21660>
- Syaj, H., Aboalhaja, N., Afifi, F., Abu-Dahab, R., Abusulieh, S., Amro, R. 2025. Phytochemistry and antiproliferative potential of a naturalized plant in Jordan: *Lavandula stoechas*. *Chemistry & Biodiversity* Apr 1, e202500181. <https://doi.org/10.1002/cbdv.202500181>. Epub ahead of print.
- Tabeshpour, J., Shakiban, D., Qobadi, A., Aghazadeh, E., Yousefsani, B.S. 2023. Cytotoxic effects of ethanolic extract of *Polypodium vulgare* on human malignant melanoma cell line. *Asian Pacific Journal of Cancer Prevention* 24(1), 275–281 <https://doi.org/10.31557/APJCP.2023.24.1.275>.
- Tsantila, E.M., Esslinger, N., Christou, M., Papageorgis, P., Neophytou, C.M. 2024. Antioxidant and anticancer activity of *Vitis vinifera* extracts in breast cell lines. *Life* 14(2), 228. <https://doi.org/10.3390/life14020228>
- UPI, The Unani Pharmacopoeia of India. 2008. 1st Ed. part-I, vol-V, Ministry of Health & Family Welfare Dept. of AYUSH, Govt. of India, New Delhi, pp. 27–28.
- Venkatesham, B., Chaithra, D., Naikodi, M.A.R., Nazeer, M., Siddiqui, A., Siddiqui, J.I., and Minhajuddin, A. 2021. Pharmacognostic evaluation, physicochemical standardization and HPTLC fingerprint analysis of pomegranate (*Punica granatum* L.) leaf and seed. *Annals of Phytomedicine* 10(2), 187–194. <https://dx.doi.org/10.21276/ap.2021.10.2.26>.
- Zahedi-Amiri, Z., Taravati, A., Hejzian, L.B. 2019. Protective effect of *Rosa damascena* against aluminum chloride-induced oxidative stress. *Biological trace element research*, 187, 120–127. <https://doi.org/10.1007/s12011-018-1348-4>.
- Zheng, Z., Zhang, L., Hou, X. 2022. Potential roles and molecular mechanisms of phytochemicals against cancer. *Food & Function* 13(18), 9208–9225. <https://doi.org/10.1039/D2FO01663J>
- Zhou, J.X., Braun, M.S., Wetterauer, P., Wetterauer, B., Wink, M. 2019. Antioxidant, cytotoxic, and antimicrobial activities of *glycyrrhiza glabra* L., *Paeonia lactiflora* Pall., and *Eriobotrya japonica* (Thunb.) Lindl. extracts. *Medicines*, 6(2), p.43. <https://doi.org/10.3390/medicines6020043>.