ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Antitumor, Antimicrobial activities and Phytochemicals Constituent of different Extracts of *Pulicaria undulata* (Forssk.) Oliver. Grown Naturally in Saudi Arabia

Khulud M Alshehri[*1], Madeha O I Ghobashy[2]

[1]Biology Department, AL-Baha University, Baljurashi, Saudi Arabia
[2]Biology Department, Faculty of Science, Tabuk University, Tabuk, Saudi Arabia

Article History:

Received on: 08 Jun 2020
Revised on: 10 Jul 2020
Accepted on: 17 Jul 2020

Keywords:

Pulicaria undulata,
Asteraceae,
GC–mass spectrometry,
HPLC,
Cytotoxicity,
Antimicrobial activity,
Herbal medicine,
MCF-7,
HCT-116,
HepG-2cell lines

ABSTRACT

Antitumor and antimicrobial resistance are a habitual global issue, which continually demands finding new natural compounds to encounter the resistance. *Pulicaria undulata* (Forssk.) Oliver. (Asteraceae family) has numerous promising medicinal properties. The recent work aimed at determination of antitumor effects of three extracts of *P. undulata* on three types of human carcinoma; HEPG-2 hepatocellular carcinoma, MCF-7 breast carcinoma and HCT-116 colon carcinoma cell lines. Anticancer activity was assessed through studying the viability of the cancer cells and apoptotic pathway. Also, antimicrobial potency of different extracts was assessed against studied human pathogens (five Gram negative bacteria, two Gram positive bacteria and yeast). The results reveal that chloroform extract has different levels of cytotoxicity toward the three types of cancer cell lines. The half maximal inhibitory concentration IC 50 value was 3.01 μg/ mL for the HepG-2, 16.4 μg/mL for the MCF-7, and 7.4 μg/ mL for HCT-116. Followed by the ethyl acetate extract which showed strong cytotoxic activity against HEPG2 with IC 50 = 12.2 μg /ml and moderate activities against MCF7 and HCT 116 and recorded (IC 50 = 26.7 and 26.4 μg /ml, respectively). While the crude methanol extract recorded the lowest cytotoxic effect against HEPG2, MCF7 and HCT 116 with (IC 50 = 51.4, 105.1 and 86.7 μg / ml, respectively). Chloroform and ethyl acetate extracts have a high antimicrobial activity more than methanol extract against the pathogens being studied. HPLC and GCMs Analysis identified numerous chemical compounds of *P. undulata* extracts with various therapeutic benefits. In conclusion, *P. undulata* has the potential to act as an antimicrobial agent against various pathogenic microbes and is a promising wild herb for the treatment of cancer.

*Corresponding Author

Name: Khulud M Alshehri
Phone:
Email: dr.k2015@hotmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i3.2790

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INTRODUCTION

Millions of people are diagnosed with different types of cancers worldwide every year. About 18.1 million new cases of cancer were estimated in 2018, and approximately 9.6 million deaths of cancer occurred. Cancer of lungs is the most prevalent type of cancer in both genders combined, thoroughly followed by female breast cancer, prostate cancer, colorectal cancer, stomach cancer, and liver cancer (Bray, 2018). Present medications can only, to a certain degree, inhibit the development of tumours in all

forms of cancer. Therefore, to resolve the numerous pharmaceutical limitations of cancer, it is essential to find alternative natural drugs for treating liver, colon, and breast cancers. Including immune system damage, several deficiencies have been found due to the severe side effects of chemical drugs produced in patients. Besides, the foremost causes of mortality and morbidity are the cancerous cell metastasis (Huang, 2017).

Nowadays, complementary therapies are also being used to treat and reduce the symptoms and pain of cancer. Natural products had been used in different parts of the world like the Kingdom of Saudi Arabia, India, and Egypt since the earliest eras as traditional remedies. Such natural products have diverse mechanisms of action such as cell growth inhibition, the disparity in the differentiation of cells and apoptosis initiation. These natural plant products have been used in the treatment of many infectious diseases and cancers, as they have antimicrobial and antitumor effects (Bourhia, 2019).

Recently the number of drug-resistant pathogens has increased substantially in medical investigation, although many new antibiotics were developed (Aslam, 2018). In this context, erroneous use of antibiotics has been attributed to the antibiotic-resistant development and the global emergence of multidrug-resistant bacteria that gradually reduce the efficacy of existing drugs resulting in treatment failure. Infectious diseases caused by antimicrobial-resistant microbes are becoming a serious problem all over the world, which leads to an increase in the morbidity and mortality of these infections (Nthulane and Patience, 2020). So, we need to explore a new active product against these multidrug-resistant microbes (MDR). In the same time, microbiologists discover a potent plant extract which can selectively antagonize with infectious microbes. These different extracts contain components of bioactive metabolites, including flavonoids, alkaloids, tannins, terpenoids, and phenolics function together in combination to compact microbial growth (Nthulane and Patience, 2020). These new classes of antimicrobial substances have been extracted from medicinal plants and strongly inhibited the growth of (MDR) organisms with novel antagonistic mechanisms. These new strategies had the potential to be used as alternative therapeutic options for the treatment of a diverse infection induced by resistant microbes (Mulani *et al.*, 2019). Recently, the commercial importance of these secondary metabolites (SMs) has given considerable attention to its growth and to explore ways to increase its production using tissue culture technology (Aslam, 2018).

As stated by the World Health Organization (WHO), around 65% of the world's population prefers traditional herbal medicines. Nonetheless, few studies on herbal drugs in the treatment of several cancers have been carried out (Jadhav, 2008). Until now, a limited number of wild plants as herbal medicines have been investigated and analyzed chemically, given the possible anticancer effect of their unique bioactive chemicals. Wild pharmaceutical plants are a good source of highly biologically active SMs, which considered as a pivotal source of active constituents with many variations in its arrangements and structural properties (Hegazy and Emam, 2015).

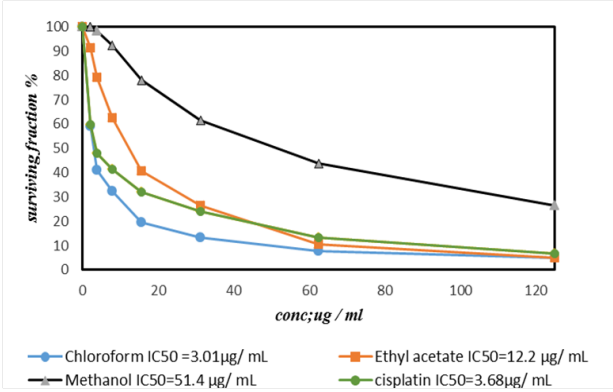

Figure 1: Cytotoxic effect of successive extract of *Pulicaria undulata* plant and Cisplatin on (HepG-2)

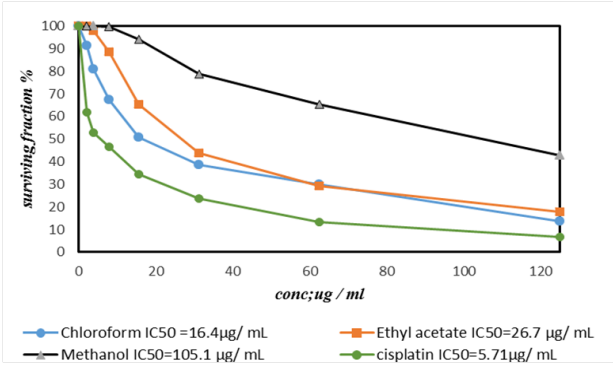

Figure 2: Cytotoxic effect of different extract of *Pulicaria undulata* plant and Cisplatin against (MCF-7).

Family Asteraceae (Compositae) is a worldwide distributed family of about 1600 genera and comprise more than 23,000 species. The genus *Pulicaria* that is belonging to Asteraceae includes about 75 species distributed widely in Asia, Africa, and Europe (Kalwij, 2012). They used in traditional medication as antihyperglycemic, and antispasmodic drugs, also they show anticancer, antioxidant and antimicrobial properties (Emam *et al.*, 2019).

Table 1: Cytotoxic activity of successive extracts of *Pulicaria undulata* plant against HepG-2, MCF-7 and HCT-116 cell lines

NO.	Extract	IC_{50} (μg/ mL)		
		HepG- 2	MCF-7	HCT-116
1	Chlorofom	3.01±0.3	16.4±1.2	7.4±0.8
2	Ethyl acetate	12.2 ±0.4	26.7±2.1	26.4 ±1.7
3	Methanol	51.4 ±1.9	105.1±4.3	86.7±3.9
4	Cisplatin	3.68 ± 0.19	5.71 ± 0.53	4.51 ± 0.72

Table 2: Antimicrobial activity of different extracts of *Pulicaria undulata*

Test microorganisms	Diameter of inhibition zone of different extracts (mm)			Diameter of inhibition zone of control (antibiotics (mm))	
	Chloro-form	Ethyl acetate	Methano	Gentamicin 32 μg	Fluconazole 32 μg
Gram-negative bacteria *Proteus mirabilis*	22 ± 0.8	16 ± 0.5	9.0 ± 0.3	20 ± 1	—
Klebsiella pneumoniae	30 ± 1.2	11 ± 0.4	11 ± 0.3	18 ± 1.3	—
Escherichia coli	NA	10 ± 0.4	12 ± 0.3	22 ± 1.1	—
Pseudomonas aeruginosa	9.0 ± 0.5	15 ± 0.6	10 ± 0.3	20 ± 1	—
Salmonella typhi	13 ± 0.7	18 ± 0.7	12 ± 0.3	19 ± 1	—
Gram-positive bacteria *Streptococcus mutans*	25 ± 0.8	19 ± 0.8	35 ± 1.2	22 ± .8	—
Staphylococcus aureus	30 ± 1.2	25 ± 0.8	20 ± 0.8	20 ± 1.2	—
Yeast *Candida albicans*	21 ± 0.8	18 ± 0.6	10 ± 0.4	—	20 ± 0.9

NA:no activity, ± SD; (Diameter on inhibition zone including well diameter of 6mm).

Table 3: Minimum Inhibition Concentration and Minimum Bactericidal Concentration (MIC and MBC) of chloroform extracts of *Pulicaria undulata*

Test microorganisms	MIC μg/ml	MBC μg/ml	MFC μg/ml
Gram-negative bacteria *Proteus mirabilis*	60	75	—
Klebsiella pneumoniae	75	100	—
Escherichia coli	NA	NA	—
Pseudomonas aeruginosa	1000	1000	—
Salmonella typhi	500	600	—
Gram -positive bacteria *Streptococcus mutans*	100	120	—
Staphylococcus aureus	50	60	—
Yeast *Candida albicans*	75	—–	100

NA: no activity, ± SD; (Diameter on inhibition zone including well diameter of 6mm).

Table 4: Minimum Inhibition Concentration and Minimum Bactericidal Concentration (MIC and MBC) of ethyl acetate extracts of *Pulicaria undulata*

Test microorganisms	MIC μg/ml	MBC μg/ml	MFC μg/ml
Gram-negative bacteria	75	100	—
Proteus mirabilis			
Klebsiella pneumoniae	1000	1000	—
Escherichia coli	500	1000	—
Pseudomonas aeruginosa	250	300	—
Salmonella typhi	150	150	—
Gram-positive bacteria	65	75	—
Streptococcus mutans			
Staphylococcus aureus	60	75	—
Yeast	100		120
Candida albicans			

Table 5: Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extracts of *Pulicaria undulata* (L.)

Test microorganisms	MIC μg/ml	MBC μg/ml	MFC μg/ml
Gram-negative bacteria	500	600	
Proteus mirabilis			
Klebsiella pneumoniae	125	200	
Escherichia coli	250	250	
Pseudomonas aeruginosa	1000	1000	
Salmonella typhi	250	500	
Gram -positive bacteria	40	50	
Streptococcus mutans			
Staphylococcus aureus	150	150	
Yeast	1000		1000
Candida albicans			

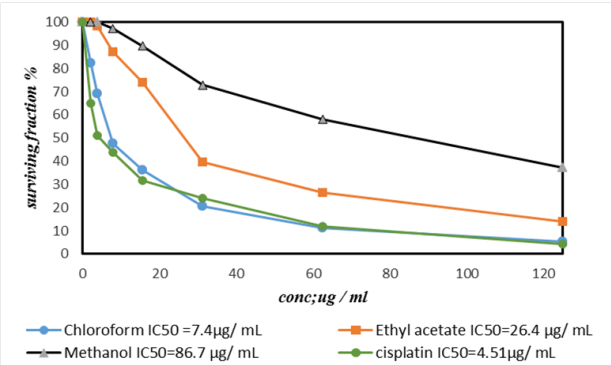

Figure 3: Cytotoxic effect of different extract of *Pulicaria undulata* plant and Cisplatin against (HCT-116)

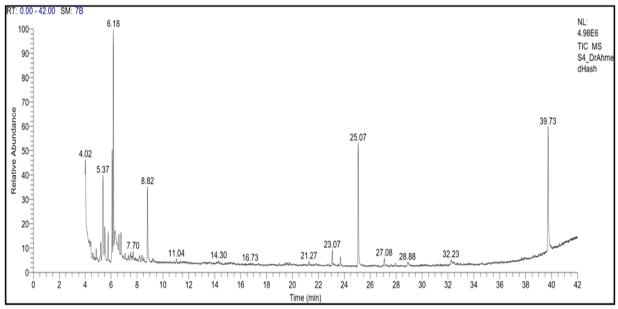

Figure 4: GC-MS analysis of chloroform extract of *Pulicaria undulata* plant

The chemical elaboration of Pulicaria species revealed the presence of various SMs, such as mono-, sesqui-, and diterpenoids, flavonoids, and phenolics (Hegazy and Emam, 2015). *Pulicaria undulata* is one of the most common annual herb or sub-shrubs grown naturally in the desert with small yellow flowers.

Based on the strong medicinal contextual of Asteraceae, *P. undulata* was selected for investigation. The recent study has been done to assess the pharmacological properties of *P. undulata*.

However, the vast gap of information's about the medicinal features of fresh *P. undulata* as fresh *P.*

Table 6: Chemical composition of chloroform extract of *Pulicaria undulata* plant by GC-MS

Peak N.	R. T	Peak area (%)	Compound name	Formula	MF
1	8.83	0.73	Tetradecane	$C_{14}H_{30}$	890
2	11.96	1.04	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	C4Cl6	887
3	17.38	3.61	Bicyclo [7.2.0] UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYLENE-, [1R-(1R*,4E,9S*)]	$C_{15}H_{24}$	948
4	18.99	3.51	alpha-Longipinene	C15H24	909
5	23.06	0.65	α-bisabolol oxide B	C15H26O2	848
6	25.07	4.23	α-bisabolol oxide A	C15H26O2	902
7	25.52	0.45	Bicyclo [4.1.0] heptan-2-ol, 1á-(3-methyl-1,3-butadienyl)-2à,6á-dimethyl-3á -acetoxy-	C16H24O3	759
8	25.68	1.57	10,12-Octadecadiynoic acid	C18H28O2	769
9	26.31	1.22	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	C21H32O2	776
10	27.08	1.00	Neophytadiene	C20H38	941
11	27.96	0.49	9,12-Octadecadienoic acid, ethyl ester	C20H36O2	798
12	28.09	4.54	Reynosin	C15H20O3	800
13	28.31	4.30	3-Heptyne, 2,2,6-trimethyl-5-chloro-6-phenyl-	C16H21Cl	816
14	28.86	1.06	Hexadecanoic acid, methyl ester	C17H34O2	893
15	30.23	4.85	Gazaniolide	C15H18O2	794
16	30.72	1.01	Thiocyanic acid, 1,1,3-trimethyl-3-phenylbutyl ester	C15H18O2	840
17	30.96	1.16	Nootkaton-11,12-epoxide	C15H22O2	762
18	31.55	0.74	Androstan-17-one, 3-ethyl-3-hydroxy-, (5à)-	C21H34O2	744
19	32.20	0.80	Cholestan-3-ol, 2-methylene-, (3á,5à)-	C28H48O	799
20	32.42	2.52	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	C20H40O	922
21	32.80	1.34	Alantolactone	C15H18O2	863
22	33.12	48.56	Tomentosin	C15H20O3	831
23	34.42	1.69	Santamarine	C15H20O3	854
24	35.64	0.82	Deoxysericealactone	C20H28O6	754
25	37.52	1.04	10,12-Tricosadiynoic acid, methyl ester	C24H40O2	757
26	37.87	0.58	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde	C23H32O	761
27	38.45	1.30	Reynosin	C15H20O3	819
28	38.69	4.65	3H-Naphtho [2,3-b] furan-2-one, 4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a, 5,6,7,9,9a-octahydro-	C15H20O3	821
29	39.72	0.56	Diisooctyl phthalate	C24H38O4	934

Table 7: Chemical composition of ethyl acetate extract of *Pulicaria undulata* plant by GC-MS

Peak N.	R. T	Peak area (%)	Compound name	Formula	MF
1	4.02	1.89	1,3-Cyclopentadiene, 5-Ethenyl-5-Methyl	C_8H_{10}	917
2	4.44	1.52	Acetic acid, pentyl ester	$C_7H_{14}O_2$	768
3	4.86	0.89	6,8-Dioxabicycl (3.2.1) Octan-3á-OL-2, 2,4,4-D4	$C_6H_6D_4O_3$	785
4	5.19	2.24	Benzene, propyl-	C_9H_{12}	886
5	5.38	10.51	Benzene, 1-Ethyl-3-Methyl-	C_9H_{12}	926
6	5.52	3.02	Benzene, 1,2,3-trimethyl-	C_9H_{12}	903
7	5.77	2.92	Benzene, 1-ethyl-3-methyl-	C_9H_{12}	873
8	6.08	9.41	Benzene, 1,2,5-trimethyl-	C_9H_{12}	954
9	6.18	17.41	Butanoic acid, butyl ester	$C_8H_{16}O_2$	968
10	6.31	2.88	Phenol	C_6H_6O	917
11	6.61	1.52	Acetic acid, hexyl ester	$C_8H_{16}O_2$	864
12	6.77	2.28	Benzene, 1,2,4-trimethyl-	C_9H_{12}	868
13	7.50	1.06	5,9-Tetradecadiyne	$C_{14}H_{22}$	816
14	8.39	1.85	6-Isopropenyl-3-methoxymethoxy-3-methyl-cyclohexene	$C_{12}H_{20}O_2$	759
15	8.82	8.24	Undecane	$C_{11}H_{24}$	920
16	23.07	1.82	2-Furanmethanol, tetrahydro-à, à, 5-trimethyl-5-(4-methyl-3-cycloh exen-1-yl)-, [2S-[2à,5á(R*)]]	$C_{15}H_{26}O_2$	860
17	23.70	1.25	(S)-2,2,6-Trimethyl-6-((S)-4-methylcyclohex-3en-1-yl) dihydro-2H-pyran-3(4H)-one	$C_{15}H_{24}O_2$	808
18	25.07	12.93	alpha. -Bisabolol oxide A	$C_{15}H_{26}O_2$	890
19	27.08	0.98	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	685
20	39.73	15.38	Diisooctyl phthalate	$C_{28}H_{48}O$	953

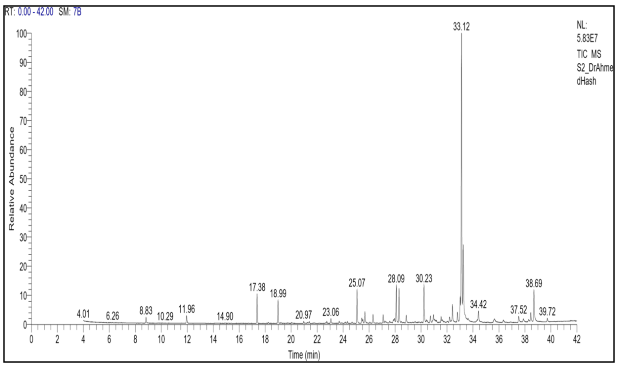

Figure 5: GC-MS analysis of Ethyl acetate extract of *Pulicaria undulata* plant

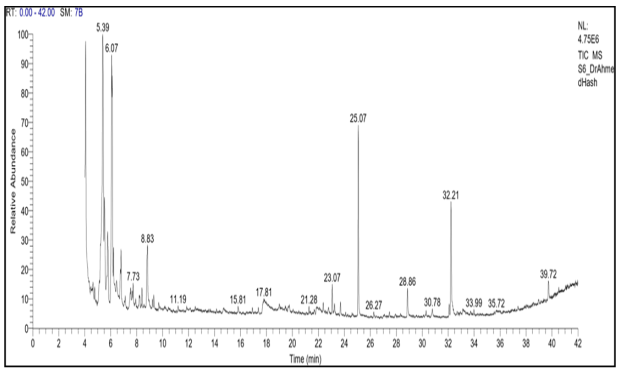

Figure 6: GC-MS analysis of Methanol extract of *Pulicaria undulata* plant

undulata is used in the utmost of traditional remedies, especially in the Saudi Arabia Kingdom. Other objectives of this study were to evaluate the antitumor and antimicrobial activities of different extracts of aerial parts of *P. undulata* and to investigate the chemical composition of each extract qualitatively and quantitatively.

Table 8: Chemical composition of methanol extract of *Pulicaria undulata* plant by GC-MS

Peak N.	R. T	Peak area (%)	Compound name	Formula	MF
1	4.07	8.35	p-Xylene	C_8H_{10}	949
2	4.77	0.57	2-Methylmalonic acid	$C_4H_6O_4$	677
3	5.20	0.62	Benzene, propyl-	C_9H_{12}	924
4	5.39	17.48	Benzene, 1-ethyl-3-methyl-	C_9H_{12}	950
5	5.52	7.38	Benzene, 1,2,3-trimethyl-	C_9H_{12}	892
6	5.77	7.12	Cumol	C_9H_{12}	896
7	6.07	20.16	Benzene, 1,3,5-trimethyl-	C_9H_{12}	950
8	6.22	1.02	7,7-Dithyl-tetracyclo [4.1.0.0(2,4).0 (3,3)] heptane	C_9H_{12}	885
9	6.45	1.04	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	638
10	7.12	0.77	Pyrimidine-4,6-dione, hexahydro-4-(3-phenyl-2-propenyl)-2-thioxo-	C_9H_{12}	672
11	7.54	1.13	Benzene, 1-methyl-3-propyl-	$C_{10}H_{14}$	869
12	7.66	0.59	Carveol	$C_{10}H_{16}O$	746
13	7.73	0.84	6,7-Dimethyl-3,5,8,8a-tetrahydro-1H-2-benzop yran	$C_{11}H_{16}O$	920
14	8.20	1.12	Spiro [3.5] nona-5,7-dien-1-one, 5,9,9-trimethyl-	$C_{12}H_{16}O$	840
15	8.41	0.96	2,3-Epoxicaran, trans-	$C_{10}H_{16}O$	803
16	8.83	5.01	Undecane	$C_{11}H_{24}$	879
17	9.22	0.47	6-Isopropenyl-3-methoxymethoxy-3-methyl-cyclohexene	$C_{12}H_{20}O_2$	809
18	9.33	0.83	p-Cymene	$C_{10}H_{14}$	715
19	22.37	0.66	8-Ketoylangenal	$C_{15}H_{20}O_2$	750
20	23.07	1.46	Bisabolol oxide B	$C_{15}H_{26}O_2$	830
21	23.25	0.64	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylid ene) pentan-2-ol	$C_{14}H_{22}O_2$	718
22	23.70	0.87	Bisabolene oxide	$C_{15}H_{24}O_2$	806
23	25.07	9.91	2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cycloh exen-1-yl)-, [3S-[3à,6à(R*)]]-	$C_{15}H_{26}O_2$	891
24	28.86	1.77	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	871
25	32.07	0.61	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	715
26	32.22	7.26	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	896
27	39.72	1.36	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	706

Table 9: Qualitative analysis of flavonoids and phenolic of different extracts of *Pulicaria undulata* by HPLC

No	Compounds	Extracts					
		Chloroform		Ethyl acetate		Methanol	
		RT.	Area %	RT.	Area %	RT.	Area %
1	Kaempferol	48.841	61.68	48.668	83.05	48.962	8.44
2	Quercitin	44.660	26.66	44.866	12.39	45.054	18.24
3	Rutin	38.464	0.22	38.664	0.69	-	-
4	Catechin	-	-	-	-	24.290	0.13
5	Gallic acid	-	-	-	-	12.469	0.10
6	Ellagic acid	-	-	-	-	-	-
7	Chlorogenic acid	-	-	27.125	3.87	-	-
8	Caffeic acid	28.752	11.25	-	-	28.662	73.09

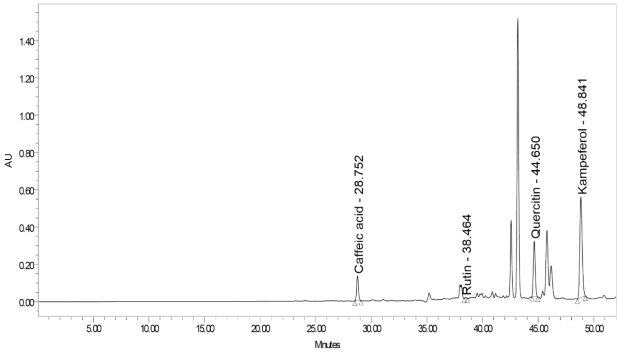

Figure 7: HPLC chromatogram of chloroform extract of *Pulicaria undulata* plant

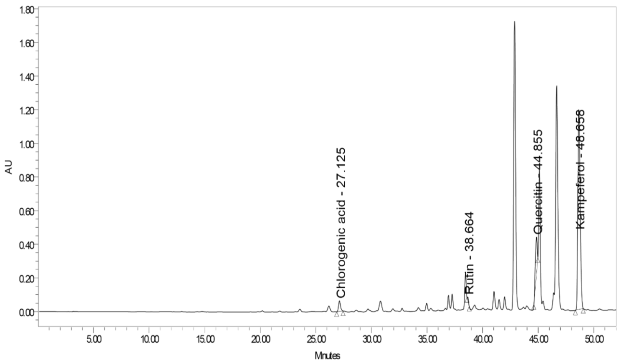

Figure 8: HPLC chromatogram of Ethyl acetate extract of *Pulicaria undulata* plant

MATERIALS AND METHODS

Chemicals and Kits

Chemicals and reagents were high analytical grade, namely Aldrich-Sigma Chemical (St. Louis MO, USA) & ADWIC, Egypt. Fetal Bovine serum, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and

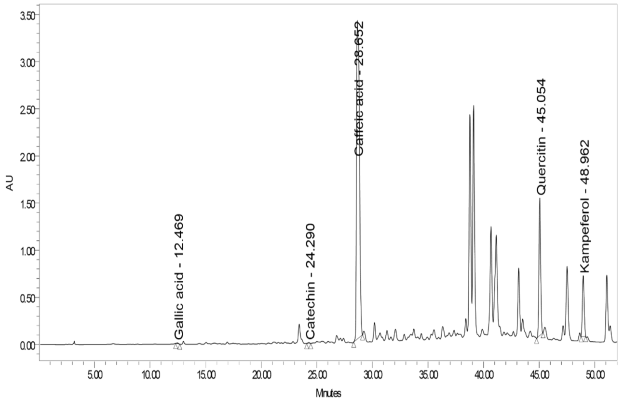

Figure 9: HPLC chromatogram of methanol extract of *Pulicaria undulata* plant

0.25% Trypsin-EDTA were purchased from Lonza (Belgium).

Plant collection and preparation of the extracts

The new aerial parts of *P. undulata* were collected at its growth period of spring season from its natural habitats in the Saudi Arabia Kingdom. The plant was air-dried at lab-temperature till constant weight, then ground to a fine powder and kept being used for different plant analysis. Two hundred grams of plant powder was successively extracted by soxhlet apparatus using different organic solvents with analytical reagent (AR) quality. These solvents were chloroform, Ethyl acetate, and finally, methanol for ten h.each extract collected separately into dry clean beakers, after that they were evaporated under reduced pressure using rota vapour apparatus at 60 °C, then were dried in desiccators for one hour and finally, all the dried residues were stored in a refrigerator at 5 ºC until the use.

In-vitro Cytotoxic Activity by MTT assay

Mammalian cell lines

HepG-2 cells (human hepatocellular carcinoma cell line), HCT-116 cells (human colon carcinoma cell line) and MCF-7 cells (human breast carcinoma cell line) were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Cell line Propagation

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50μg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were sub-cultured two to three times a week.

Cytotoxicity evaluation using viability assay

The antitumor activity for different extracts and Cisplatin drug as positive control evaluated according to the method described by Mosmann (1983). By MTT assay the number of viable cells was determined, and the percentage of viability was calculated as [(ODt/ODc)] x100% where it is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of the untreated cell.

The survival curve of each tumour cell line after treatment with the specified drug was plotted from the relation between surviving cells and drug concentration. By GraphPad Prism software (San Diego, CA. USA), the 50% inhibitory concentration (IC_{50}) was estimated from graphic plots of the dose-response curve for each level.

Antimicrobial activity

The antimicrobial effectiveness of the chloroform, ethyl acetate, and methanol extracts was determined using the agar well diffusion method (Murray *et al.*, 1999).

The prepared extracts were examined for its antibacterial and antifungal activities against studied pathogenic microorganisms (Gram-negative bacteria (GNB): *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhi*, Gram-positive bacteria (GPB): *Streptococcus mutans*, *Staphylococcus aureus* and yeast: *Candida albicans*).

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and the Minimum Fungicidal Concentration (MFC)

MIC, MBC and MFC of the all studied extracts were carried out according to Murray *et al.* (1999) using modified Broth dilution assay with the help of Spectrophotometer at 595 nm in mg/ml.

Chemical Composition Evaluation

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of various crude extracts was performed using Trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 μm film thickness) with the same condition as recorded by (Hashmi *et al.*, 2013). The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

Qualitative Determination of Flavonoids and phenolics Using HPLC

High-performance liquid chromatography (HPLC) technique using Waters 2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector., set at flow 1 ml/min. Autosampler, degasser, column compartment set at 35ºC and variable wavelength detector set at 280 nm, column: Hypersil C18 Thermo 5μm, 250x4.6 mm was used and the mobile phase: Buffer (0.1 % phosphoric acid in water) and methanol. A stock solution of 8 different standards in methanol was prepared. Each of the standards was filtered using a 0.22 μm syringe filter then 10μl were injected. The prepared concentrations were Kampferol 0.4mg/ml, Gallic acid 1.2 mg/ml, Ellagic acid 0.4mg/ml, Chlorogenic acid 0.7mg/ml, Catechin 0.7mg/ml, Quercitin 0.3mg/ml, caffeic acid 1mg/ml and rutin 1mg/ml.

Statistical analysis

The results were analyzed using a two-way analysis of variance (ANOVA). All statistical investigations were carried out using SPSS 18.0 software. The findings were reported as standard error (SE)$\pm$ of three replicates, and statistical significance was set as p-value$\leq$0.05.

RESULTS AND DISCUSSION

Cytotoxic activity

The common therapies as radiation, chemotherapy, and surgery had limited efficiency, so the mortality rate among cancer patients is high (Xu *et al.*, 2009). Recently, the researcher has been interested in using of crude plant extracts as natural productsor a combination of different phytochemicals for cancer therapy; this course is based upon the synergistic effect of the various plant metabolites in the crude extract and its multiple points of the intervention of such extracts.

According to the previous protocols of the American National Cancer Institute NCI (Boyd, 1997), the

results expressed strong when IC_{50} less than 20 μg/ml and moderate activities when IC_{50}=21 -50 μg/ml. It was observed from the obtained results in a Table 1 and Figure 1, Figure 2 and Figure 3 that, all extracts of *P. undulata* achieved a cytotoxic effect against HEPG2, MCF7, HCT 116. While chloroform extract was had strong cytotoxic activity against HEPG2, MCF7 and HCT 116 with (IC_{50}=3.01, 16.4 and 7.4 μg/ ml, respectively) followed by the ethyl acetate extract which showed strong cytotoxic activity against HEPG2 with IC_{50}= 12.2 μg /ml and moderate activities against MCF7 and HCT 116 and recorded (IC_{50}= 26.7 and 26.4 μg /ml, respectively). While the crude methanol extract recorded the lowest cytotoxic effect against HEPG2, MCF7 and HCT 116 with (IC_{50} =51.4, 105.1 and 86.7 μg / ml, respectively). AnEgyptian study by (Emam *et al.*, 2019) recorded that methanol crude extract of *P. undulata* has cytotoxic activity against HEPG2 with IC_{50}= 27.7 mg /ml and Hussien *et al.* (2016) reported that the crude extract (CH_2Cl_2/MeOH) of P. undulata showed excellent cytotoxic activity against both MCF-7 cells and HEPG2 cells with IC_{50} 41.6 and 40.7 μg/ml respectively.

Antimicrobial activity of *P. undulata*

The different extracts (chloroform, ethyl acetate, and methanol) of *P.undulata* exhibit antimicrobial activity against test microorganisms, GNB: *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhi*, GPB: *Streptococcus mutans*, *Staphylococcus aureus*, yeast: *Candida albicans*) as shown in Table 2.

P. undulata extracts (chloroform, ethyl acetate, and methanol) show significant antimicrobial activity. Chloroform extract records high activity against examined microorganisms except for *E. coli* (no activity).

But the most significant activity against *S. aureus* and *K. pneumonia* (30 mm inhibition zone) and lowest activity against *P. aeruginosa* (9.0 mm. inhibition zone).

Ethyl acetate extract showing mild activity against tested pathogens where *S. aureus* shows high sensitivity for extract about 25 mm diameter of inhibition zone. At the same time, *E. coli* exhibits resistance for extract 10 mm (inhibition zone).

Methanol extract showing the lowest activity against tested microbes where the highest activity of the extract against *S. mutans* 35 mm of clear zone, while *P. mirabilis* shows resistance for extract 9.0 mm inhibition zone.

S. mutans and *S. aureus* were more sensitive microbes for all extracts, and on the other hand, *E. coli* was more resistance for all extracts.

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungal concentration (MFC)

(MIC), (MBC) and (MFC) of *P. undulata*, different extracts are recorded in the Table 3, Table 4 and Table 5. From the results of Table 3, Table 4 and Table 5 which revealed that the MIC & MBC are going in two parallel directions. In all extracts, the best MIC will be followed by the best MBC. Table 3, discus the MIC & MBC effects of the chloroform extract on the different studied pathogens. It is clear that it has excellent MIC effect against *S. aureus, P. mirabilis* and *K. pneumonia* (50, 60, & 75 μg/ml, respectively). This results followed by the same thing of MBC effects against the same organisms (60, 75, & 100 μg/ml, respectively). On the other hand, *E. coli* has no MIC nor MBC activities.

In the same manner, as the Table 3, Table 4 results explain the effects of the ethyl acetate extract MIC & MBC against the studied pathogens. Ethyl acetate extract clearly shows perfect MIC & MBC effects against *S. aureus, S. mutans* and *P. mirabilis* (60, 65, & 75 μg/ml, respectively) followed by lower effect against the remaining pathogens. Table 5, show the effect of the *P. undulata* methanolic extract on the different studied pathogenic microbes. The results show that MIC & MBC are weaker than the previous two extracts (chloroform & ethyl acetate extracts) except *S. mutans* strain which show high sensitivity to the methanolic extract (MIC; 40 & MBC; 50 μg/ml, respectively).

These results of Table 3, Table 4 and Table 5 are in agreement with the study of (Touati *et al.*, 2018), who recorded that chloroform and methanol extracts of *Pulicaria odora* have potent antimicrobial activity against Gram-positive bacteria *S. aureus* and *B. subtilis* while Gram-negative *P. aeruginosa* and *E. coli* were more resistance for all studied extracts. They reported that MIC and MBC values from 1.4 to 2 mg/ml, and this result confirms our results. Another researcher confirmed that different extracts (ethanol, Petroleum ether, ethyl acetate, and methanol) of *P. undulata* collected from Omdurman, Sudan, exhibited antimicrobial activity on all test microbes Gram-negative and Gram-positive at the same time (Gram-positive: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236), and gram-negative: *Escherichia coli* (ATCC 25922), *Proteus Vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella para typhi* B (0650) and *Klebsiella pneumoniae* (ATCC 1312)) (El-Kamali and Mahjoub, 2009). The most potent activity (about 30 mm) was recorded for Petroleum ether

toward *B. subtilis*, while water extracts not possess activity against all tested strains. They reported that ethanol, ethyl acetate, and methanol extracts exhibit activity on all examined microbes ranged from 15 to 26 mm diameter of inhibition zone, while MIC and MBC for those extracts ranged from 3.125 to 100 μg/ml. Additionally, (Ajaib, 2015) determined, that *P. undulata* was collected from Lahore, Pakistan and extracted with various solvent. Their solvent has shown antimicrobial action toward tested microorganisms (gram-positive: *S. aureus* and *B. subtilis*, Gram-negative: *E. coli* and *P. aeruginosa*, and fungi: *A. niger* and *F. solani*). They recorded, that all extracts (Petroleum ether, chloroform, methanol, and water) exhibit the high significant value of antimicrobial activity against all examined microbes where value extended from 17 to 44 mm diameter of inhibition zone for all extracts.

In the same context, the Table 3, Table 4 and Table 5 show that the MFC results of the studied extracts show high antifungal activity (MFC) against the studied *Candida albicans* for chloroform and ethyl acetate extracts (21 $\pm$ 0.8 and 18 $\pm$ 0.6 mm inhibition zone diameter, respectively) and low antifungal activity of methanolic extract (10 $\pm$ 0.4 mm inhibition zone diameter) comparing to the standard fluconazole antibiotics (20 mm inhibition zone diameter). It agrees with (Helal, 2019) who reported that methanolic extract of *P. undulata* showing antifungal activity for some fungal strains, for example, *C. Albicans* 20 mm diameter of inhibition zone and the highest activity toward *M. boulardii* about 32 mm.

Phytochemical evaluation

It was performed for qualitative and quantitative detection of various chemical constituents in *P. undulata* which aid in tracing the presence of an active entity that elicit a significant biological response of the plant. The mass spectrum of the unknown component was compared with the spectrum of the known element stored in the National Institute Standard and Technology (NIST) library. The compound name, probability, molecular formula, molecular weight and peak area of the test materials were recorded. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. GC-MS analysis of the chloroform extract revealed the presence of 29 compounds (Figure 4 & Table 6) the major components were tomentosin (48.56%), is a natural sesquiterpene lactone. Many medicinal plants from the Asteraceae family are rich by sesquiterpenes lactones which have cytotoxic and anticancer properties (Hegazy, 2015). Sesquiterpenes lactones are potentially selective toward tumour and cancer stem cells by targeting specific signalling pathways, which make them lead compounds in cancer clinical trials (Zhang *et al.*, 2005). Tomentosin showed antibacterial and antifungal effects (Masoumian and Zandi, 2017) and *in vitro* antiproliferative activity on various human cancer cell lines (Hegazy, 2015).

GC-MS analysis of the ethyl acetate extract revealed the presence of 20 compounds (Figure 5 & Table 7) the major components were Butanoic acid, butyl ester (17.41%), Diisooctyl phthalate (15.38%) and alpha.-Bisabolol oxide A (12.93%) and Benzene, 1-Ethyl-3-Methyl- (10.51%), while GC-MS analysis of the methanol extract revealed the presence of 27 compounds (Figure 6 & Table 8) the major components were Benzene, 1,3,5-trimethyl- (20.16%) and Benzene, 1-ethyl-3-methyl- (17.48%).

It is difficult to characterize every compound present in the crude extract to elucidate its structure, due to the diversity and complexity of natural phenolic compounds (Surveswaran *et al.*, 2007), qualitative estimation for some phenolic and flavonoids compounds for a different successive extract of *P. undulata* was observed at the Table 9. The chloroform extract contains kaempferol, quercetin, caffeic acid and rutin Figure 7, the ethyl acetate extract contains kaempferol, quercetin, Chlorogenic acid and rutin Figure 8 and the methanolic extract contain caffeic acid, quercetin, kaempferol, catechin and gallicacid Figure 9. Flavonoids and phenolic components have been reported as antioxidants, anticancer, antibacterial, cardioprotective agents, anti-inflammation, immune system promoting, and skin protection from UV radiation, and interesting candidate for pharmaceutical and medical applications (Tungmunnithum *et al.*, 2018). Many studies have suggested that flavonoids like rutin, kaempferol, quercetin, apigenin etc. are well-known for its anti-inflammatory, anti-allergic, anti-thrombotic, hepatoprotective, anti-spasmodic and anticancer properties (Tungmunnithum *et al.*, 2018).

CONCLUSION

Our study showed that all different extracts of *P. undulata* possess marked and moderate cytotoxic activity against different three cell line using MTT assay, besides Antimicrobial test of *P. undulata* proved that chloroform and ethyl acetate extracts exhibited a high value of lethal activity against most of the examined human pathogens. Also, the value of MIC, MBC and MFC activities of *P. undulata* extracts can be used as natural therapeutic compounds against a wide range of pathogenic microor-

ganisms, instead of the traditional commonly used antibiotics. These activities may be due to its abundance of many biologically active phytochemical compounds which provide a useful document for further study on our plant to detect its impact on another cancer type *in vivo* study.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Dr Ahmed Hashem, Assistant Professor. Ain shams University, Department of Botany, for the constant support during this research. I also wish to express my thanks for Dr MarwaAbdel-salam Ibrahim, Researcher, Desert research centre, Medicinal and Aromatic Plants Department, for her patience, motivation, and profuse knowledge.

Funding Support

Nil.

Conflict of Interest

The author declares that there is no conflict of interest.

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