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

Khulud M Alshehri¹, Madeha O I Ghobashy²

¹Biology Department, AL-Baha University, Baljurashi, Saudi Arabia
²Biology 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-2 cell 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. A considerable decline in cancer cell rates has been linked to increasing in concentration of plant extract. The half maximal inhibitory concentration IC₅₀ 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 IC₅₀ = 12.2 μg/ml and moderate activities against MCF7 and HCT 116 and recorded (IC₅₀ = 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 (IC₅₀ = 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.

**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).

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 anti spasmodic drugs, also they show anticancer, antioxidant and antimicrobial properties (Emam et al., 2019). The chemical elaboration of Pulicaria species
Table 1: Cytotoxic activity of successive extracts of *Pulicaria undulata* plant against HepG-2, MCF-7 and HCT-116 cell lines

| NO. | Extract     | HepG-2 IC₅₀ (µg/mL) | MCF-7 IC₅₀ (µg/mL) | HCT-116 IC₅₀ (µg/mL) |
|-----|-------------|----------------------|--------------------|----------------------|
| 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)) |
|---------------------|--------------------------------------------------------|---------------------------------------------------------|
|                     | Chloroform | Ethyl acetate | Methanol | Gentamicin | Fluconazole |
| **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 ± 0.8 | — |
| *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** |           |           |           |
| *Proteus mirabilis*     | 75        | 100       | —         |
| *Klebsiella pneumoniae* | 1000      | 1000      | —         |
| *Escherichia coli*      | 500       | 1000      | —         |
| *Pseudomonas aeruginosa*| 250       | 300       | —         |
| *Salmonella typhi*      | 150       | 150       | —         |
| **Gram-positive bacteria** |           |           |           |
| *Streptococcus mutans*  | 65        | 75        | —         |
| *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** |           |           |           |
| *Proteus mirabilis*     | 500       | 600       |           |
| *Klebsiella pneumoniae* | 125       | 200       |           |
| *Escherichia coli*      | 250       | 250       |           |
| *Pseudomonas aeruginosa*| 1000      | 1000      |           |
| *Salmonella typhi*      | 250       | 500       |           |
| **Gram-positive bacteria** |           |           |           |
| *Streptococcus mutans*  | 40        | 50        |           |
| *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

revealed the presence of various SMs, such as monoterpens, sesqui-, and diterpenoids, flavonoids, and phenolics (Hegazy and Emam, 2015). *Pulicaria undulata* is one of the most common annual herb or subshrubs 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 about the medicinal features of fresh *P. undulata* as fresh *P. undulata* is used in the utmost of traditional remedies, especially in...
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- | C_{4}C_{16} | 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 | C_{15}H_{24}O_{3} | 744 |
| 5       | 23.06 | 0.65          | α-bisabolol oxide B | C_{20}H_{38}O_{2} | 798 |
| 6       | 25.07 | 4.23          | α-bisabolol oxide A | C_{15}H_{26}O_{2} | 902 |
| 7       | 25.52 | 0.45          | Bicyclo [4.1.0] heptan-2-ol, 1α-(3-methyl-1,3-butadienyl)-2α,6α-dimethyl-3α-acetoxy- | C_{16}H_{24}O_{3} | 759 |
| 8       | 25.68 | 1.57          | 10,12-Octadecadiynoic acid | C_{18}H_{28}O_{2} | 769 |
| 9       | 26.31 | 1.22          | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)- | C_{21}H_{34}O_{2} | 776 |
| 10      | 27.08 | 1.00          | Neophytadiene | C_{20}H_{38}O_{2} | 798 |
| 11      | 27.96 | 0.49          | 9,12-Octadecadienoic acid, ethyl ester | C_{15}H_{26}O_{2} | 800 |
| 12      | 28.09 | 4.54          | Reynosin | C_{16}H_{21}Cl | 816 |
| 13      | 28.31 | 4.30          | 3-Heptyne, 2,2,6-trimethyl-5-chloro-6-phenyl- | C_{16}H_{21}Cl | 816 |
| 14      | 28.86 | 1.06          | Hexadecanoic acid, methyl ester | C_{17}H_{34}O_{2} | 893 |
| 15      | 30.23 | 4.85          | Gazaniolide | C_{15}H_{18}O_{2} | 794 |
| 16      | 30.72 | 1.01          | Thiocyanic acid, 1,1,3-trimethyl-3-phenylbutyl ester | C_{16}H_{21}Cl | 840 |
| 17      | 30.96 | 1.16          | Nootkaton-11,12-epoxide | C_{15}H_{22}O_{2} | 762 |
| 18      | 31.55 | 0.74          | Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)- | C_{21}H_{34}O_{2} | 744 |
| 19      | 32.20 | 0.80          | Cholestan-3-ol, 2-methylene-, (3α,5α)- | C_{18}H_{40}O_{2} | 799 |
| 20      | 32.42 | 2.52          | 3,7,11,15-Tetramethyl-2-hexadecen-1-OL | C_{20}H_{40}O_{2} | 922 |
| 21      | 32.80 | 1.34          | Alantolactone | C_{15}H_{18}O_{2} | 863 |
| 22      | 33.12 | 48.56         | Tomentosin | C_{15}H_{20}O_{3} | 831 |
| 23      | 34.42 | 1.69          | Santamarine | C_{15}H_{26}O_{2} | 854 |
| 24      | 35.64 | 0.82          | Deoxysericealactone | C_{15}H_{22}O_{2} | 754 |
| 25      | 37.52 | 1.04          | 10,12-Tricosadiynoic acid, methyl ester | C_{16}H_{20}O_{3} | 757 |
| 26      | 37.87 | 0.58          | 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-triienyl] cyclohex-1-en-1-carboxaldehyde | C_{23}H_{32}O_{2} | 761 |
| 27      | 38.45 | 1.30          | Reynosin | C_{15}H_{20}O_{3} | 819 |
| 28      | 38.69 | 4.65          | 3H-Naphtho [2,3-b] furan-2-one, 4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9a-octahydro- | C_{15}H_{20}O_{3} | 821 |
| 29      | 39.72 | 0.56          | Diisooctyl phthalate | C_{24}H_{38}O_{2} | 934 |

© International Journal of Research in Pharmaceutical Sciences
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, Ethenyl-5-Methyl | C₈H₁₀ | 917 |
| 2       | 4.44  | 1.52          | Acetic acid, pentyl ester | C₇H₁₄O₂ | 768 |
| 3       | 4.86  | 0.89          | 6,8-Dioxabicycl (3.2.1) Octan-3a-OL-2, 2,4,4-D₄ | C₆H₆D₄O₃ | 785 |
| 4       | 5.19  | 2.24          | Benzene, propyl- | C₉H₁₂ | 886 |
| 5       | 5.38  | 10.51         | Benzene, 1-Ethyl-3-Methyl- | C₉H₁₂ | 926 |
| 6       | 5.52  | 3.02          | Benzene, 1,2,3-trimethyl- | C₉H₁₂ | 903 |
| 7       | 5.77  | 2.92          | Benzene, 1-ethyl-3-methyl- | C₉H₁₂ | 873 |
| 8       | 6.08  | 9.41          | Benzene, 1,2,5-trimethyl- | C₉H₁₂ | 954 |
| 9       | 6.18  | 17.41         | Butanoic acid, butyl ester | C₈H₁₆O₂ | 968 |
| 10      | 6.31  | 2.88          | Phenol | C₆H₆O | 917 |
| 11      | 6.61  | 1.52          | Acetic acid, hexyl ester | C₆H₁₆O₂ | 864 |
| 12      | 6.77  | 2.28          | Benzene, 1,2,4-trimethyl- | C₉H₁₂ | 868 |
| 13      | 7.50  | 1.06          | 5,9-Tetradecadiyne | C₁₄H₂₂ | 816 |
| 14      | 8.39  | 1.85          | 6-Isopropenyl-3-methoxymethoxy-3-methylcyclohexene | C₁₂H₂₀O₂ | 759 |
| 15      | 8.82  | 8.24          | Undecane | C₁₁H₂₄ | 920 |
| 16      | 23.07 | 1.82          | 2-Furanmethanol, tetrahydro-2, 2, 5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2à,5à(R*)]] | C₁₅H₂₄O₂ | 860 |
| 17      | 23.70 | 1.25          | (S)-2,2,6-Trimethyl-6-((S)-4-methylcyclohex-3-en-1-yl)-dihydro-2H-pyran-3(4H)-one | C₁₅H₂₄O₂ | 808 |
| 18      | 25.07 | 12.93         | alpha-Bisabolol oxide A | C₁₅H₂₆O₂ | 890 |
| 19      | 27.08 | 0.98          | 9-Octadecenoic acid (Z)- | C₁₈H₃₀O₂ | 685 |
| 20      | 39.73 | 15.38         | Diisooctyl phthalate | C₂₈H₄₈O | 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

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                                          | C₈H₁₀   | 949 |
| 2      | 4.77  | 0.57          | 2-Methylmalonic acid                             | C₆H₁₀O₄ | 677 |
| 3      | 5.20  | 0.62          | Benzene, propyl-                                 | C₉H₁₂   | 924 |
| 4      | 5.39  | 17.48         | Benzene, 1-ethyl-3-methyl-                        | C₉H₁₂   | 950 |
| 5      | 5.52  | 7.38          | Benzene, 1,2,3-trimethyl-                         | C₉H₁₂   | 892 |
| 6      | 5.77  | 7.12          | Cumol                                             | C₉H₁₂   | 896 |
| 7      | 6.07  | 20.16         | Benzene, 1,3,5-trimethyl-                         | C₉H₁₂   | 950 |
| 8      | 6.22  | 1.02          | 7,7-Dithyl-tetracyclo [4.1.0.0(2,4).0(3,3)] heptane | C₉H₁₂   | 885 |
| 9      | 6.45  | 1.04          | 10-Heptadecan-8-ynoic acid, methyl ester; (E)-    | C₁₈H₃₀O₂ | 638 |
| 10     | 7.12  | 0.77          | Pyrimidine-4,6-dione, hexahydro-4-(3-phenyl-2-propenyl)-2-thioxo- | C₉H₁₂   | 672 |
| 11     | 7.54  | 1.13          | Benzene, 1-methyl-3-propyl-                       | C₁₀H₁₄  | 869 |
| 12     | 7.66  | 0.59          | Carveol                                           | C₁₀H₁₄O  | 746 |
| 13     | 7.73  | 0.84          | 6,7-Dimethyl-3,5,8,8a-tetrahydro-1H-2-benzopyran  | C₁₁H₁₆O  | 920 |
| 14     | 8.20  | 1.12          | Spiro [3.5] nona-5,7-dien-1-one, 5,9,9-trimethyl-  | C₁₂H₁₆O  | 840 |
| 15     | 8.41  | 0.96          | 2,3-Epoxicaran, trans-                           | C₁₀H₁₆O  | 803 |
| 16     | 8.83  | 5.01          | Undecane                                         | C₁₁H₂₄   | 879 |
| 17     | 9.22  | 0.47          | 6-Isopropenyl-3-methoxymethoxy-3-methyl-cyclohexene | C₁₂H₂₀O₂ | 809 |
| 18     | 9.33  | 0.83          | p-Cymene                                         | C₁₀H₁₄  | 715 |
| 19     | 22.37 | 0.66          | 8-Ketoylangenal                                   | C₁₃H₂₀O₂ | 750 |
| 20     | 23.07 | 1.46          | Bisabolol oxide B                                | C₁₃H₂₀O₂ | 830 |
| 21     | 23.25 | 0.64          | 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol | C₁₄H₂₂O₂ | 718 |
| 22     | 23.70 | 0.87          | Bisabolene oxide                                 | C₁₃H₂₄O₂ | 806 |
| 23     | 25.07 | 9.91          | 2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohex-1-en-1-yl)-, [3S-[3a,6a(R*)]]- | C₁₃H₂₆O₂ | 891 |
| 24     | 28.86 | 1.77          | Hexadecanoic acid, methyl ester                   | C₁₇H₃₄O₂ | 871 |
| 25     | 32.07 | 0.61          | Linoleic acid ethyl ester                        | C₂₀H₃₀O₂ | 715 |
| 26     | 32.22 | 7.26          | 9-Octadecenoic acid (Z)-, methyl ester            | C₁₉H₃₀O₂ | 896 |
| 27     | 39.72 | 1.36          | 1,2-Benzenedicarboxylic acid                    | C₂₄H₃₈O₄ | 706 |
Table 9: Qualitative analysis of flavonoids and phenolic of different extracts of *Pulicaria undulata* by HPLC

| No | Compound          | 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                |

**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 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 °C 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 37°C in a humidified atmosphere with 5% CO2 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)] x 100% 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 (IC50) 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 (GBP): 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 35°C and variable wavelength detector set at 280 nm, column: Hypersil C18 Thermo 5μ, 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) ± of three replicates, and statistical significance was set as p-value ≤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 products or 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 IC50 less than 20 μg/ml and moderate activities when IC50=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 cyto-
toxic effect against HEPG2, MCF7, HCT 116. While
chloroform extract was had strong cytotoxic activity
against HEPG2, MCF7 and HCT 116 with (IC<sub>50</sub>=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<sub>50</sub>=12.2 µg/ml and
moderate activities against MCF7 and HCT 116 and
recorded (IC<sub>50</sub>= 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<sub>50</sub>=14.1, 105.1 and 26.7 µg/ml,
respectively). An Egyptian study by (Emam et al., 2019)
recorded that methanol crude extract of *P. undulata*
has cytotoxic activity against HEPG2 with IC<sub>50</sub>= 27.7
mg/ml and Hussien et al. (2017) reported that the
crude extract (CH<sub>2</sub>Cl<sub>2</sub>/MeOH) of *P. undulata*
showed excellent cytotoxic activity against both MCF-7 cells
and HEPG2 cells with IC<sub>50</sub> 41.6 and 40.7 µg/ml
respectively.

**Antimicrobial activity of *P. undulata***

The different extracts (chloroform, ethyl acetate, and methanol) of *Pundulata* exhibit antimicro-
bial 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
least activity against *P. aeruginosa* (9.0 mm. inhibition
zone).

Ethyl acetate extract showing mild activity against
tested pathogens where *S. aureus* shows high sensi-
tivity 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 inhi-
bition 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), min-
imum 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, dis-
cuss 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 odorosa* have potent antimicro-
bial 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: *Staphylo-
coccus 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 ± 0.8 and 18 ± 0.6 mm inhibition zone diameter, respectively) and low antifungal activity of methanolic extract (10 ± 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. bouillardii 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%), Dixoocyt 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 microorganisms, 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

© International Journal of Research in Pharmaceutical Sciences 4899
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 Marwa Abdel-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.

REFERENCES

Ajaib, M. 2015. Pulicaria Undulata: A Potential Phytochemical, Antimicrobial and Antioxidant Source. *Journal of the Chemical Society of Pakistan*, 37(3):559–66.

Aslam, B. 2018. Antibiotic Resistance: A Rundown of a Global Crisis. *Infection and Drug Resistance*, 11:1645–58.

Bourhia, M. 2019. Ethnopharmacological Survey of Herbal Remedies Used for the Treatment of Cancer in the Greater Casablanca-Morocco. *Evidence-Based Complementary and Alternative Medicine*.

Boyd, M. R. 1997. The NCI In Vitro Anticancer Drug Discovery Screen. *Anticancer Drug Development Guide: Preclinical Screening*, pages 23–42.

Bray, F. 2018. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, 68(6):394–424.

El-Kamali, H., Mahjoub, S. 2009. Antibacterial Activity of Francoeuria Crispa, Pulicaria Undulata, Ziziphus Spina-Christi and Cucurbita Pepo Against Seven Standard Pathogenic Bacteria. *Ethnobotanical Leaflets*, (6).

Emam, M. A., Khattab, H. I., Hegazy, M. G. 2019. Assessment of anticancer activity of Pulicaria undulata on hepatocellular carcinoma HepG2 cell line. *Tumor Biology*, 41(10):101042831988008–101042831988008.

Hashmi, L. S. A., Hossain, M. A., Weli, A. M., Al-Riyami, Q., AlSabahi, J. N. 2013. Gas chromatography–mass spectrometry analysis of different organic crude extracts from the local medicinal plant of Thymus vulgaris L. *Asian Pacific Journal of Trop-ical Biomedicine*, 3(1):69–73.

Hegazy, M.-E. F. 2015. Rare Hydroperoxyxyl Guaianolide Sesquiterpenes from Pulicaria Undulata. *Phytochemistry Letters*, 12:177–81.

Hegazy, M. G. A., Emam, M. A. 2015. Ethanolic Extract of Trigonella Foenum Graecum Attenuates Cisplatin-Induced Nephro- and Hepatotoxicities in Rats. *Cellular and Molecular Biology*, 12(7):81–87.

Halal, I. M. 2019. Antimicrobial Efficiency of Essential Oils from Traditional Medicinal Plants of Asir Region, Saudi Arabia, over Drug Resistant Isolates. *BioMed Research International*, pages 8928306–8928306.

Huang, C.-Y. 2017. A Review on the Effects of Current Chemotherapy Drugs and Natural Agents in Treating Non-Small Cell Lung Cancer. *BioMedicine*, 7(4).

Hussien, T. A., El-toumy, S. A., Hassan, H. M., Hetta, M. H. 2016. CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF SECONDARY METABOLITES FROM PULICARIA UNDULATA. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(9):150–150.

Jadhav, D. 2008. Medicinal Plants of India. volume 2. Scientific Publishers. ISBN: 9788172335472.

Kalwij, J. M. 2012. Review of ‘The Plant List, a working list of all plant species’. *Journal of Vegetation Science*, 23(5):998–1002.

Masoumian, M., Zandi, M. 2017. Antimicrobial Activity of Some Medicinal Plant Extracts against Multidrug Resistant Bacteria. *Zahedan Journal of Research in Medical Sciences*, 19(11).

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2):55–63.

Mulani, M. S., Kamble, E. E., Kumkar, S. N., Tawre, M. S., Pardesi, K. R. 2019. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Frontiers in Microbiology*, 10:539–539.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., Yolken, R. H., American Society for Microbiology 1999. Manual of Clinical Microbiology. Washington, D.C. ASM Press.

Nthulane, N., Patience 2020. Antimicrobial and Anti-Inflammatory Activities of Selected Medicinal Plants against Pathogens Causing Sexually Transmitted Infections. *Journal of Herbmed Pharmacology*, 9(2):130–167.

Surveswaran, S., Cai, Y., Corke, H., Sun, M. 2007. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food
Touati, N., Saidani, K., Boudries, H., Hammiche, H., Ouazene, N., Bedjou, F. 2018. Antibacterial activity of phenolic compounds of Pulicaria odora, wild plant in northern Algeria. *International Food Research Journal*, (5):25–25.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., Yangsabai, A. 2018. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines*, 5(3).

Xu, J., Liu, X. S., Zhou, S.-F., Wei, M. Q. 2009. Combination of Immunotherapy with Anaerobic Bacteria for Immunogenetic Therapy of Solid Tumours. *Gene Therapy and Molecular Biology*, 13(1):36–52.

Zhang, S., Won, Y.-K., Ong, C.-N., Shen, H.-M., Ingenta connect 2005. Anti-Cancer Potential of Sesquiterpene Lactones: Bioactivity and Molecular Mechanisms. 5(3):239–249.