Applied Biological Research 24(1): 413-419; (2022)
DOI: 10.5958/0974-4517.2022.00049.0

ANALYSIS OF CHEMICAL COMPOSITION BY GC/MS AND ASSESSMENT OF ANTICANCER ACTIVITY OF RHUBARB (*Rheum ribes* Linn.) STALKS

Raad A Kaskoos[1] and Javed Ahamad[2,*]

[1]*Faculty of Pharmacy, Hawler Medical University, Kurdistan Region, Erbil (Iraq)*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Tishk International University, Kurdistan Region, Erbil (Iraq)*
[*]e-mail: *jas.hamdard@gmail.com*, *javed.ahamad@tiu.edu.iq*

(Received 30 June, 2022; accepted 12 October, 2022)

ABSTRACT

***Rheum ribes* Linn. (Polygonaceae) is an important medicinal plant abundantly growing in Iraqi Kurdistan. In Kurdish society this plant is traditionally used as laxative for the treatment of constipation. The aim of present study was to characterize the chemical compounds present in the ethanolic extract of *R. ribes* and evaluate its anticancer activity. The chemical characterization of ethanolic extract of *R. ribes* was done by GC/MS. Its anticancer activity was determined by MTT assay using human breast cancer (MCF-7) cell lines. Sixty-two chemical compounds were identified by GC/MS analysis. Hexatriacontane (11.2%), heptadecane-2,6,10,15-tetramethyl (5.53%), 7-hexadecenal, (z)- (4.63%), vitamin E (3.85%), 3,7,11,15-tetramethylhexadec-2-en-1-yl dodecanoate (3.67%), and tetratriacontane (3.65%) were observed as the major chemical compounds in the ethanolic extract. The ethanolic extract showed a time- and concentration-dependent cytotoxicity with IC_{50} value of 312.31 ± 4.19 μg mL^{-1}. The present study revealed the anticancer potential of *R. ribes* extract and provides a scientific basis for its possible use as anticancer remedy.**

Keywords: Breast cancer, GC/MS analysis, *Rheum ribes*, MTT assay

INTRODUCTION

The rhubarb (*Rheum* species) is an important medicinal plant belonging to the family Polygonaceae. The common species of rhubarb are *Rheum ribes, R. palmatum, R. australe, R. nanum, R. tibeticum, R. nobile, R. webbianum,* and *R. turkestanicum* (Keser *et al.*, 2020). Most of the species in genus *Rheum* are found in Asian countries *viz*., Turkey, Iran, and Iraq (Abu-Irmaileh, and Afifi, 2003). In Kurdistan region of Iraq, *R. ribes,* the most abundant species, is consumed as a vegetable. In both traditional and modern medicines, *Rheum* spp. have shown to possess a number of therapeutic benefits, including the treatment of cancer, diabetes, insect bites, relapsing fevers, diarrhea, and constipation (Mohtashami *et al*., 2021). Traditionally, *R. ribes* is used as laxative in Kurdish society (Keshavarzi *et al.*, 2021). *R. ribes* has been reported as potent antidiabetic plant (Kasabri *et al.,* 2011), and also possesses antioxidant and antimicrobial activities (Bazzas *et al*., 2005; Özturk *et al.*, 2007; Alaadin *et al.*, 2007).

Cancer is a condition in which the body's cells proliferate in uncontrolled manner. Breast cancer is the 2nd most prevalent kind of cancer in women in the US, after skin cancer. Although the number of breast cancer deaths has decreased over time, it is still the 2nd most common leading cause of death among women (CDC, 2022). In USA alone, about 264,000 women are diagnosed with breast cancer each year and of these about 42,000 die every year (CDC, 2022). The available treatment for breast cancer includes surgery, chemotherapy, hormonal therapy, and radiation therapy; and these therapies are associated with severe side effects. There is an urgent need to explore alternative therapy for breast cancer with less side effects (Najibullah *et al.*, 2022). Natural products are known for their anticancer role in human, and several potent anticancer medicines have been discovered from natural products such as vincristine, vinblastine, paclitaxel, docetaxel, irinotecan, etc. (Fabricant and Farnsworth, 2001; Newman and Cragg, 2016; Ahamad *et al.*, 2019). *R. ribes* has shown beneficial anticancer potential in *in-vitro* assays (Achakzai *et al.*, 2019; Ayan *et al.*, 2020) collected from different places. The present study was aimed to characterize the chemical composition of ethanolic extract of *R. ribes* by GC/MS and assess its anticancer activity by MTT assay using MCF-7 (human breast cancer) cell lines.

MATERIALS AND METHODS

Plant material and chemicals

The fresh stalks of *Rheum ribes* (1 kg) were purchased from local vegetable market in Erbil, Iraq. A voucher specimen of plant material, after proper identification, was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Tishk International University, Erbil, Iraq. MCF-7 (human breast cancer) cell line was purchased from NCCS, Pune (India). DMEM (Dulbecco’s modified eagle medium), FBS (fetal bovine serum) and antibiotic solution were purchased from Gibco (USA), DMSO (dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) from Sigma USA, 1X PBS from Himedia (India) and 96 well tissue culture plate and wash beaker from Tarson (India).

Preparation of plant extract

The fresh stalks of *Rheum ribes* were dried in shade under proper ventilation conditions for a week. After drying the plant samples were pulverized to a coarse powder in a mixture grinder. The coarse powder of *R. ribes* (100 g) was extracted in an ultrasonic bath for 30 min at 40ºC using ethanol (250 mL). Then the extract was filtered and concentrated under reduced pressure using Rota evaporator (Buchi. Switzerland). The dried extract was stored in a refrigerator at 4ºC until further use.

GC/MS analysis of R. ribes extract

The chemical composition of ethanolic extract of *R. ribes* stalks was determined by GC/MS method (Ahamad *et al.,* 2022). The ethanolic extract of *R. ribes* was analyzed on Agilent Bench Top GC-MS (Agilent Technologies, Wilmington, USA) equipment using a DB-5 glass capillary column with specification of 30 m × 0.25 mm i.d.; film thickness of 0.25 µm. The carrier gas was helium with flow rate fixed at 0.95 mL min^{-1}. The oven temperature was set at 50°C for 1 min and then isothermally maintained at 300°C for 2 min, while injector port temperature was 280°C. The split ratio was retained at 1:10 for injecting the ethanolic extract (0.1 µL, in DMSO). Data was collected at 70 eV with 1.5 sec scanning durations in the mass range of 50-1000 amu and a run time of 31 min. Chem station software was used for analyzing chromatography and mass spectra.

The individual chemical constituents were identified by comparing their Kovats Index (K.I.) with earlier reports (Babushok *et al.,* 2011) and by mass fragmentation pattern of spectra obtained from GC/MS. The compounds were compared with those stored in spectrometer database of NIST, NBS 54 K.L and WILEY8 libraries, and published literatures (Ali, 2001; Adams, 2007; Achakzai *et al.*, 2019). The area of each peak was used to calculate the composition of each component.

Assessment of anticancer activity by MTT assay

The *R. ribes* extract was evaluated for *in vitro* cytotoxicity, using MCF-7 cells by MTT assay (Kaskoos *et al.,* 2021). Briefly, the MCF-7 cell lines were cultured in liquid DMEM medium (Kaskoos *et al.*, 2021) supplemented with 10% FBS, 100 ug penicillin mL^{-1} and 100 µg streptomycin mL^{-1} and maintained under an atmosphere of 5% CO_2 at 37ºC. The cultured MCF-7 cells were harvested by trypsinization, pooled in 15 mL tube and then plated at a density of 1×10^5 cells mL^{-1} well^{-1} (200 µL) into 96-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48 h at 37°C. The wells were washed with sterile PBS and treated with various concentrations of *R. ribes* extracts in serum-free DMEM medium. Each treatment was replicated 3 times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After incubation, MTT (20 µL of 5 mg mL^{-1}) was added into each well and the cells again incubated for 2-4 h until purple precipitates was clearly visible under an inverted microscope. Finally, the medium along with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µL). To dissolve formazan crystals, DMSO (100 µL) was added, and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo-Fisher Scientific, USA) and percent cell viability and IC_{50} values were calculated using Graph Pad Prism 6.0 software (USA).

RESULTS AND DISCUSSION

GC/MS analysis of R. ribes extract

Ultrasonication was used for the extraction of *R. ribes* stalks, because ultrasonication is fast method and requires less time and solvents (Ahamad *et al.*, 2019). The *R. ribes* stalks were extracted with ethanol and the extraction yield was 6.74 ± 0.84% w/w based upon dry plant mass. The ethanolic extract of *R. ribes* was further analyzed by GC/MS, and 62 chemical compounds were identified that constituted 80.81% of total constituents (Table 1; Fig. 1). Hexatriacontane (11.21%), heptadecane-2,6,10,15-tetramethyl (5.53%), 7-hexadecenal, (z)- (4.63%), vitamin E (3.85%), 3,7,11,15-tetramethylhexadec-2-en-1-yl dodecanoate (3.67%), silane, trimethyl[[(3*β*)-stigmast-5-en-3-yl]oxy]- (3.66%), tetratriacontane (3.65%), clionasterol (3.34%), hentriacontane (3.12%), β-sitosterol acetate (3.10%), γ-tocopherol (2.57%), dotriacontane (2.34%), and 24-norursa-3,12-diene (2.12%) were identified as the major chemical compounds in the extract (Table 1). The GC/MS analysis of butanolic fraction of *R. ribes* was performed by Achakzai *et al.* (2019) and in this study the chemical compounds were identified on the basis simple comparison of retention time and *m/z* ratio. In present study, the chemical compounds were identified based on Kovats index and fragmentation pattern stored in library, which provides more reliable data.

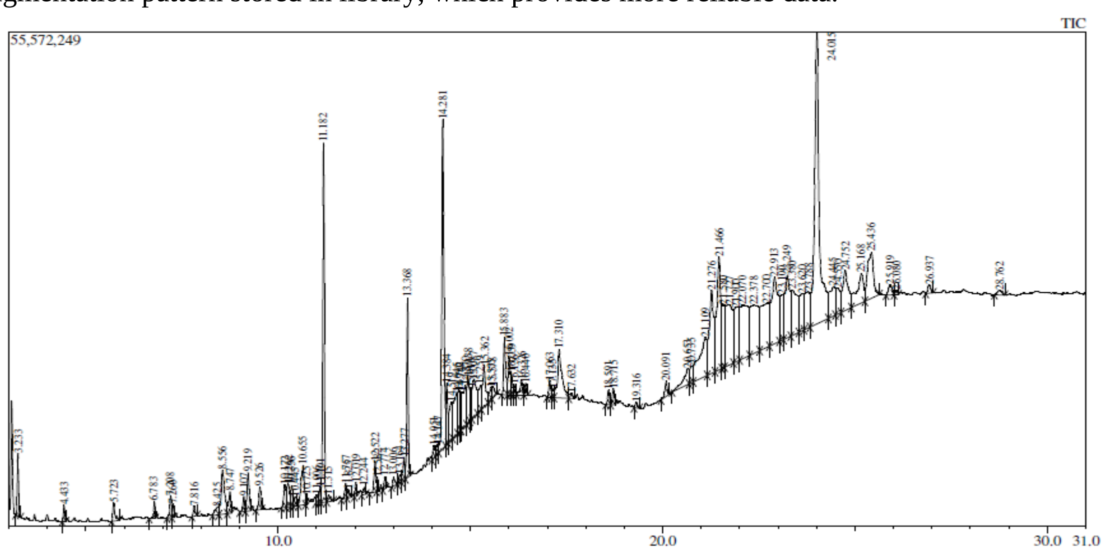

Fig. 1: GC/MS spectrum of ethanolic extract of *R. ribes* stalks

Table 1: Chemical compounds identified by GC/MS in the ethanolic extract of *R. ribes* stalks

Chemical compounds	Retention time (min)	Kovats index	Composition (%)
Ethane, 1,1-diethoxy-	3.233	730	0.64
2,3-Dimethyl-2-pentanol	4.433	779	0.18
Furfural	5.723	794	0.29
2-Methylundecane	6.783	1165	0.22
5,7-Dimethylundecane	8.425	1190	0.24
5-Hydroxymethylfurfural	8.556	1224	1.28
2-Methoxy-4-vinylphenol	8.747	1313	0.38
2,3,5,8-Tetramethyldecane	9.219	1318	0.66
Dodecane, 4,6-dimethyl	10.172	1325	0.41
Capric acid, ethyl ester	10.262	1391	0.55
Ethyl decanoate	10.356	1397	0.29
Undecylenic acid	10.445	1421	0.19
Isoeugenol	10.655	1429	1.04
Phthalic acid, diethyl ester	10.725	1603	0.11
Humulol	11.006	1609	0.14
Dichloroacetic acid, undec-2-enyl ester	11.101	1775	0.22
7-Hexadecenal, (*Z*)	11.182	1798	4.63
9-Octadecene	12.244	1803	0.13
(*E*)-11-Hexadecenal	12.522	1810	0.48
Neophytadiene	12.582	1827	0.15
Hexadecanal	13.006	1830	0.14
2-Pentadecanone, 6,10,14-trimethyl	13.164	1842	0.12
24-Noroleana-3,12-diene	13.277	1866	0.38
24-Norursa-3,12-diene	13.368	1879	2.12
Nonadecane	14.147	1900	0.20
Heptadecane-2,6,10,15-tetramethyl	14.281	1914	5.53
Decyl propylcarbamate	14.384	1926	0.88
Methyl palmitate	14.519	1927	1.14
Isophytol	14.645	1944	1.32
2,4,5-*t*-Isobutyl ester	14.702	1956	0.78
Ethyl palmitate	14.749	1968	0.21
(*Z*)-13-Octadecenal	14.870	1985	1.41
(*E*)-11-Hexadecenoic acid, ethyl ester	14.938	1974	0.90
1,2-Hexadecanediol	15.016	2031	0.47
2,13-Octadecadien-1-ol	15.087	2075	1.19
E,*E*-2,13-Octadecadien-1-ol acetate	15.259	2078	0.67
Z-Phytol	15.362	2114	1.06
2,3-Benzofluorene	15.578	2157	0.22
1-Docosene	15.883	2192	0.93
Isopentyl palmitate	16.002	2260	0.58
1-Eicosanol	16.059	2276	0.31
Ethyl nonadecanoate	16.136	2278	0.15
Tetracosane	17.135	2400	0.15
Cardanol monoene	17.310	2479	1.79
tans-11-Tetradecenoic acid	18.591	2567	0.22
Ethyl docosanoate	18.715	2596	0.28
Dioctyl phthalate	20.091	2642	0.30
Diethyl Icosanedioate	20.653	2791	0.73
1-Hexacosanol	20.735	2852	0.45
γ-Tocopherol	21.109	2987	2.57

Hentriacontane	21.276	3100	3.12
Vitamin E	21.466	3112	3.85
Stigmasta-5,22-dien-3-ol	21.580	3138	1.20
3,7,11,15-Tetramethylhexadec-2-en-1-yl dodecanoate	21.727	3160	3.67
Dotriacontane	21.900	3200	2.34
Silane, trimethyl[[(3*β*)-stigmast-5-en-3-yl]oxy]-	22.070	3349	3.66
Clionasterol	22.378	3351	3.34
β-Sitosterol acetate	22.700	3357	3.10
Tetratriacontane	22.913	3400	3.65
β-Sitosterol	23.100	3408	1.23
Hexatriacontane	24.015	3600	11.21
5*α*-Stigmastane-3,6-dione	24.550	3601	1.00

Anticancer activity of R. ribes

The cytotoxic activity of ethanolic extract of *R. ribes* stalks was assessed against MCF-7 (human breast cancer cells) by MTT assay method. The ethanolic extract showed time (24 h) and concentration (0.5-500 µg mL^{-1}) dependent cytotoxicity. After 24 h of treatment with *R. ribes*, the cell viability of A549 cell lines ranged from 98.03 to 48.95 at concentrations of 0.5-500 µg mL^{-1}, respectively (Fig. 2 & 3; Table 2).

For MCF-7 cell lines, the IC_{50} value of *R. ribes* ethanolic extract after 24 h incubation was 312.3 ± 4.2 µg mL^{-1}. Even when the incubation was extended, the untreated MCF-7 cells maintained their original morphology and showed intimate contact with each other (Fig. 3). After 24 h treatment with ethanolic extract, the MCF-7 cells lost their natural form. The elongated spindle-shaped morphology of MCF-7 cell lines vanished. Suspension cells (dead cells) were discovered after the treatment was extended to 48 h, and more suspension cells were observed after 24 h (Fig. 3B-G). Higher plants contain novel phytochemicals and since ancient times make contribution to human health in the form of food medicine and spices. Medicinal plants are the source of drugs for the treatment of chronic diseases such as cancer, diabetes, malaria, arthritis, etc. Several anticancer phytochemicals (e.g. vincristine, vincristine, vinblastine, irinotecan, paclitaxel, docetaxel, and topotecan, etc.) have been discovered and included in modern treatment of chronic diseases like cancer, diabetes, malaria, arthritis, etc. Several anticancer phytochemicals (e.g., docetaxel, vincristine, vinblastine, paclitaxel, topotecan, and irinotecan, etc.) have been discovered and included in modern system of medicine for treatment of many kinds of cancer (Ahmad and Ahamad, 2020). MTT assay has emerged as a method of choice for screening of anticancer potential of medicinal plants using cell lines as it is economical, easy and provides reliable data (Kaskoos *et al.*, 2021). The MTT assay now

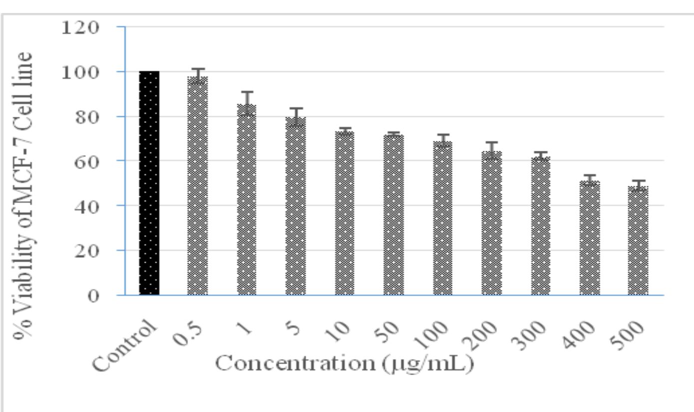

Fig. 2: The cytotoxicity of MCF-7 cell line due to the ethanolic extract of *R. ribes* stalks by MTT assay (Data presented as mean of triplicate determinations ± SD)

Table 2: Cell viability produced by ethanolic extract of *R. ribes* stalks

Concentration (µg mL^{-1})	Cell viability (%)
0.0 (control)	100
0.5	98.03 ± 3.17
1.0	85.92 ± 5.14
5.0	79.75 ± 3.84
10.0	73.33 ± 1.46
50.0	71.99 ± 0.57
100.0	69.13 ± 2.75
200.0	64.63 ± 3.62
300.0	62.33 ± 1.77
400.0	51.51 ± 2.11
500.0	48.95 ± 2.33
IC_{50} value	312.31 ± 4.19

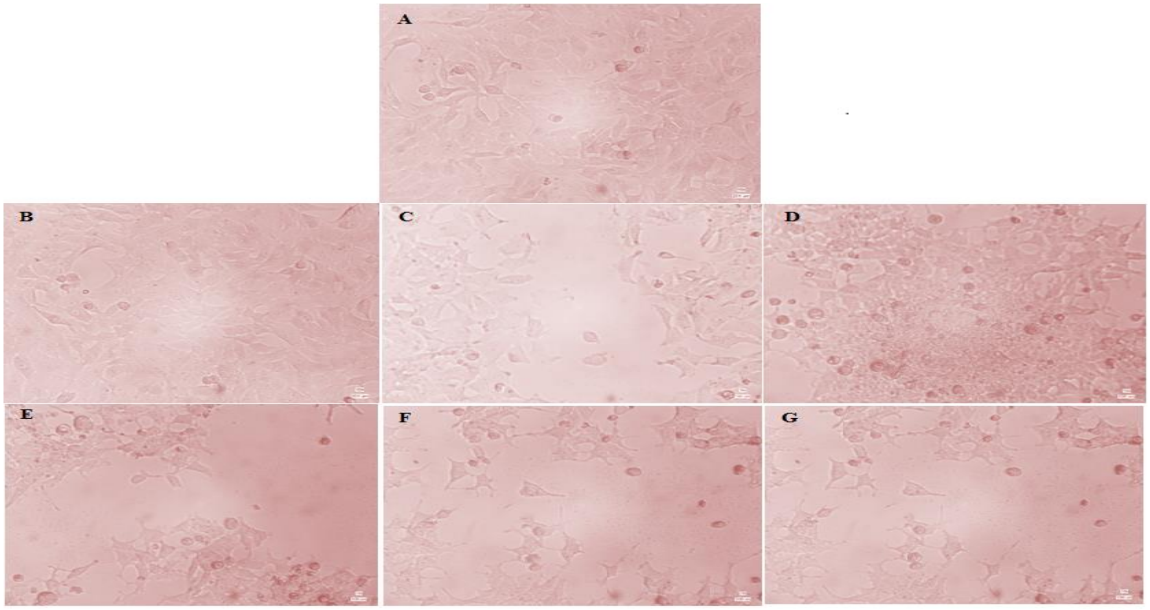

Fig. 3: Cell cytotoxicity of MCF-7 cell lines due to the ethanolic extract of *R. ribes* stalks; A): control cells; B-G): *R. ribes* stalks ethanolic extract wherein B: 1 µg mL^{-1}, C: 10 µg mL^{-1}, D: 50 µg mL^{-1}, E: 100 µ mL^{-1}, F: 300 µg mL^{-1}, and G: 500 µg mL^{-1}.

days used for screening of medicinal plants for cytotoxic potential (Ahamad *et al.*, 2019; Najibullah *et al.*, 2022). The study provides preliminary data on *R. ribes* ethanolic extract that it has potent cytotoxic activity against MCF-7 cell lines. This finding suggests that the reduction in cell viability occurs due to treatment with *R. ribes* ethanolic extract. The cytotoxic activity produced by *R. ribes* may be due to the presence of phytochemicals in its ethanolic extract (Talib and Mahasneh, 2011).

Conclusion: Rhubarb contains anthraquinone glycosides and is mainly used for the treatment of constipation. In Kurdistan region of Iraq *R. ribes* is abundantly found and traditionally used as a laxative. The present study provides preliminary data on cytotoxic potential of *R. ribes*. It provides the basis for further study on *R. ribes* for isolation and characterization of new phytochemicals as an anticancer agent. GC/MS analysis resulted in the identification of 62 chemical compounds, of which hexatriacontane, 7-hexadecenal, (*Z*), vitamin E, 3,7,11,15-tetramethylhexadec-2-en-1-yl dodecanoate, and tetratriacontane were the major chemical compounds in the ethanolic extract of *R. ribes*. This study further enriched existing knowledge of Rhubarb about its chemical compounds.

REFERENCE

Abu-Irmaileh, B.E. and Afifi, F.U. 2003. Herbal medicine in Jordan with special emphasis on commonly used herbs. *Journal of Ethnopharmacology*, **89**: 193-197.

Achakzai, J.K., Panezai, A.M., Kakar, M.A., Kakar, A.M., Kakar, S., Khan, J., Khan, N.Y., Khilji, I. and Tareen, A.K. 2019. *In vitro* anticancer MCF-7, anti-inflammatory, and brine shrimp lethality assay (BSLA) and GC-MS analysis of whole plant butanol fraction of *Rheum ribes* (WBFRR). *BioMed Research International*, 3264846. [*doi: 10.1155/2019/3264846*].

Adams, R.P. 2007. *Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry* (4th edn.). Allured Publishing, Carol Stream, Illinois, USA.

Ahamad, J., Omer, A.Y., Majid, D.A., Khidr, T.M. and Jameel, S.Y. 2022. Essential oil characterization in clove (*Syzygium aromaticum* Linn.) by GC-MS and detection of its adulteration by ATR-FTIR method. *Applied Biological Research*, **24(**3): 309-316.

Ahamad, J., Uthirapathy, S., Ameen, M.S.M. an, Anwar, E.T. 2019. Essential oil composition and antidiabetic, anticancer activity of *Rosmarinus officinalis* L. leaves from Erbil (Iraq). *Journal of Essential Oil Bearing Plants*, **22**(6): 1544-1553.

Ahmad, J. and Ahamad, J. 2020. *Bioactive Phytochemicals: Drug Discovery to Product Development* (1st edn.). Bentham Science Publisher, Sharjah, UAE.

Alaadin, M.A., Khateeb, E.H. and Jäger, A.K. 2007. Antibacterial activity of the Iraqi *Rheum ribes* root. *Pharmaceutical Biology*, **45**(9): 688-690.

Ali, M. 2001. *Techniques in Terpenoid Identification*. Birla Publication, Delhi, India.

Ayan, I.C., Çetinkaya, S., Dursun, H.G. and Süntar, I. 2020. Bioactive compounds of *Rheum ribes* L. and its anticancerogenic effect via induction of apoptosis and miR-200 family expression in human colorectal cancer cells. *Nutrition and Cancer*, **73**(7): 1228-1243.

Babushok, V.I., Linstrom, P.J. and Zenkevich, I.G. 2011. Retention indices for frequently reported compounds of plant essential oils. *Journal of Physical and Chemical Reference Data*, **40**(4): 043101. [*DOI: 10.1063/1.3653552*].

Bazzas, B.S.F., Khajehkaramadin, M. and Shokooheizadeh, H.R. 2005. *In vitro* antimicrobial activity of *Rheum ribes* extract obtained from various plant parts against clinical isolates of gram-negative pathogens. *Iranian Journal of Pharmaceutical Research*, **2**: 87-91.

Centre for Disease Control and Prevention (CDC). 2022. *Basic Information About Breast Cancer*. [*https://www.cdc.gov/cancer/breast/basic info/index.htm*].

Fabricant, D.S. and Farnsworth, N.R. 2001. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*., **109** (supplement 1): 69-75.

Farnsworth, N.R. 1990. The role of ethnopharmacology in drug development. *Ciba Foundation Symposium*, **154**: 2-11.

Kasabri, V., Afifi, F.U. and Hamdan, I. 2011. *In vitro* and *in vivo* acute antihyperglycemic effects of five selected indigenous plants from Jordan used in traditional medicine. *Journal of Ethnopharmacology*, **133**(2): 888-896.

Kaskoos, R.A., Ahamad, J. and Uthirapathy, S. 2021. Chemical composition and cytotoxic activity of *Pistacia atlantica* var. *kurdica* fruits. *ARO-The Scientific Journal of Koya University*, **9**(2): 91 -95.

Keser, S., Keser, F., Karatepe, M., Kaygili, O., Tekin, S., Turkoglu, I., Demir, E., Yilmaz, O., Kirbag, S. and Sandal, S. 2020. Bioactive contents, *in vitro* antiradical, antimicrobial and cytotoxic properties of rhubarb (*Rheum ribes* L.) extracts. *Natural Product Research*, **34**(23): 3353-3357.

Keshavarzi, Z., Shakeri, F., Maghool, F., Jamialahmadi, T., Johnston, T.P. and Sahebkar, A. 2021. A review on the phytochemistry, pharmacology, and therapeutic effects of *Rheum ribes*. *Natural Products and Human Diseases*, **1328**: 447-461.

Mohtashami, L., Amiri, M.S., Ayati, Z., Ramezani, M., Jamialahmadi, T., Emami, S.A. and Sahebkar, A. 2021. Ethnobotanical uses, phytochemistry and pharmacology of different *Rheum* species (Polygonaceae): A review. *Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health*, **1308:** 309-352.

Najibullah, S.N.M., Ahamad, J., Sultana, S. and Zafar, A. 2022. Chemical characterization and evaluation of anticancer activity of *Pistacia terebinthus* Linn. fruits essential oil. *Journal of Essential Oil Bearing Plants*, **25**(1): 180-187.

Newman, D.J. and Cragg, G.M. 2016. Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Product*, **79**: 629-661.

Özturk, M., Aydoðmuº-Özturk F., Duru M.E. and Topcu, G. 2007. Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant. *Food Chemistry*, **103**: 623-630.

Talib, W.H. and Mahasneh, A.M. 2011. Antiproliferative activity of plant extracts used against cancer in traditional medicine. *Scientia Pharmaceutica*, **78**: 33-45.