Cytotoxic Activity and Phytochemical Analysis of *Artemisia haussknechtii* Boiss

Sajjad Nasseri, Mohammad-Reza Delnavaz  , Farshad H. Shirazi  and Faraz Mojab

1Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
3Department of Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

**Abstract**

Cytotoxic activity of crude extract and fractions (petroleum ether, dichloromethane, and n-butanol) of *Artemisia haussknechtii* aerial parts was investigated by MTT assay. Dichloromethane fraction showed the highest cytotoxic effect on MCF-7 cell line (IC$_{50}$ = 297.17 ± 7.99 $\mu$g/mL). Phytochemical analysis of the most effective fraction was carried out using normal phase column chromatography (CC) to get eight sub-fractions (A-H). Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) were used for further purification. Four known compounds with cytotoxic effects on cancer cell lines were isolated from the most active fraction, including 5-Hydroxy-3',4',6,7-tetramethoxyflavone (eupatilin 7-methyl ether), 5-hydroxy 3,3',4',6,7-pentamethoxy-flavone (artemetin), 6-methoxy-7-hydroxycoumarin (scopoletin), and methyl caffeate. Structure elucidation of isolated compounds was done using spectroscopic techniques, including ESIMS and 1D-NMR ($^1$H and $^{13}$C). Cytotoxic activity of *A. haussknechtii* is probably due to coumarin and flavonoid compounds.

**Keywords:** *Artemisia haussknechtii*, MTT Assay, Phytochemical Analysis, 5-Hydroxy-3',4',6,7-tetramethoxyflavone, Artemetin, Scopoletin, Methyl Caffeate

1. **Background**

The genus *Artemisia*, with more than 500 species, is the largest member of the Compositae (Asteraceae) family, distributed in different areas of Asia, Europe, and North America (1). The inhibitory effects of essential oils, crude extracts, and different fractions of *Artemisia* against the growth of cancer cell lines have been reported (2). The studies have demonstrated that secondary metabolites, mainly flavonoids, terpenoids, and coumarins, are responsible for the cytotoxic effects in the genus *Artemisia* (3-6). Essential oil of *A. persica* and *A. turcomanica* inhibited the MCF-7 cell line time and dose-dependently (7). Essential oil of *A. annua* showed cytotoxic activity on SMMC-7721 cells via inducing apoptosis (8). Essential oil of *A. lavandulaefolia* exhibited antiproliferative activity against HeLa cells by activating caspase 3 (9). Essential oil of *A. vulgaris* induced apoptosis in HL-60 leukemic cells and showed anticancer activity with cytochrome C releasing and activating caspases-3, 8, and 9 (10). Dichloromethane extract of *A. biennis* showed the most effective cytotoxic activity on K562 and HL-60 cancer cell lines by apoptosis induction (11). Similar results have been obtained for *A. ciniformis*, *A. turanica*, and *A. armeniaca* on human cancer cell lines (12-14). Select fractions obtained from *A. ciniformis* and *A. biennis* were assessed for their cytotoxic activity on B16/P10, PC3, and MCF-7 cells and revealed promising results as potential sources of cytotoxic phytochemicals (15). A study on extracts of three samples of *A. khorassanica* for their cytotoxic activity against AGS, HELA, HT-29, and MCF-7 exhibited that dichloromethane extract was most effective on cancer cell lines. Furthermore, MCF-7 was the most sensitive among other cell lines (16).

*Artemisia haussknechtii* Boiss., with Persian names Dermeneh Zagrosi and Dermeneh Sakhreie, is one of the 34 species of *Artemisia* that grows in the west of Iran (17). A study on its crude extracts and essential oils has demonstrated in vitro antifungal (18), antibacterial, antioxidant (19), and insecticidal activities (20). Also, camphor and 1,8-cineole have been reported as significant constituents in the volatile oil. Other components included cis-davanone, 4-terpineol, linalool, beta-fenchyl alcohol, and borneol (21, 22).
2. Objectives

Due to the importance of the *Artemisia* genus for inhibiting the growth of different cancer cell lines, this study investigated the cytotoxicity effect of their crude extract and fractions. In addition, the purification and structure elucidation of compounds responsible for cytotoxic effects of the most effective fraction were done.

3. Methods

3.1. Plant Material

The aerial parts of *A. haussknechtii* Boiss. were collected from Kurdistan province (Salavat Abad mountain pass), Iran, in November 2018. The sample was identified by Alireza Dolatyari. The voucher specimen (No. 320) was deposited in the Herbarium of the Iranian Biological Research Center, Tehran, Iran.

3.2. Extraction and Fractionation

Shade-dried and grained aerial parts of *A. haussknechtii* (800 g) were macerated by 70% MeOH/H<sub>2</sub>O (4 × 8 L × 3 days). The crude extract (100 g) was fractionated via liquid-liquid extraction. The crude extract was dispersed in H<sub>2</sub>O, and three solvents (petroleum ether (40:60), dichloromethane, and n-butanol) were used to yield 3, 11, and 65 g of each fraction, respectively. All samples were concentrated using a rotary evaporator under reduced pressure and temperature below 45°C.

3.3. General Experimental Procedures

The chromatographic process was performed using an analytical HPLC system (Shimadzu LC 10A, Tokyo, Japan) equipped with a photodiode array detector (PDA) and an analytical HPLC system (Shimadzu Lc 10A, Tokyo, Japan) equipped with a photodiode array detector (PDA) and an analytical HPLC system (Shimadzu Lc 10A, Tokyo, Japan).

Further purification of *C* was performed on TLC sheets (silica gel 60 F<sub>254</sub>, Merck, Germany) and Chem lab (Belgium) with laboratory and HPLC grades.

3.4. Cell Culture Conditions

Human breast adenocarcinoma MCF-7 cell line (ATCC No. HTB-22) was purchased from the Pasteur Institute (Tehran, Iran) and maintained at 37°C in a humidified atmosphere (90%) containing 5% CO<sub>2</sub>. The cells were seeded in the RPMI-1640 medium with 10% (v/v) heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin (Gibco, USA).

3.5. Cytotoxicity Assay

The cytotoxic effects of crude extract and fractions on the MCF-7 cell line were evaluated by MTT assay. First, 180 µL of medium containing MCF-7 cells was seeded on 96-well plates (approximately 5 × 10<sup>3</sup> cells/well). Each well was treated with a specific concentration of samples (20 µL) after 24 h incubation (37°C and 5% CO<sub>2</sub>). All samples and media were removed 48 h later. Then, 20 µL MTT (5 mg/mL) was added to each well, and the plates were further incubated for 3 h at 37°C. Finally, 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve formazan crystals. All experiments were repeated three times for each concentration. The optical density (OD) was measured on a microplate reader (BioTek Instruments, USA) at 570 nm. The inhibitory rate of cell proliferation was calculated according to the following formula: Growth inhibition (%) = (A<sub>control</sub> - A<sub>treated</sub>) / A<sub>control</sub> × 100

Where A<sub>control</sub> is the absorbance of the control group (containing no samples) and A<sub>treated</sub> indicates absorbance of the sample at 570 nm. The results were reported for each sample as IC<sub>50</sub> (µg/mL).

3.6. Purification and Structure Elucidation

The chromatographic process of the most effective fraction based on cytotoxic results was performed with column chromatography (CC) (L = 100 cm and D = 5.5 cm). A portion of dichloromethane fraction (10 g) moved on silica gel normal phase (230 - 400 mesh ASTM, Merck, Germany) column and eluted with the increasing amount of ethyl acetate in chloroform (1:9 - 10:0) to get eight subfractions (A-H) (Figure 1).

Further purification of *C* was performed on TLC sheets and mobile phase chloroform: ethyl acetate (9:1) to get compounds 1 and 2 (R<sub>f</sub> = 0.65, 5 mg, and R<sub>f</sub> = 0.72, 2 mg, respectively). Purification of D by analytical HPLC (mobile phase: 0 - 40, MeOH from 20% to 80% in H<sub>2</sub>O; 40 - 45 min, 80% MeOH in H<sub>2</sub>O; 45 - 46 min, 80% MeOH in H<sub>2</sub>O to 100% MeOH; 46 - 50 min, MeOH 100%; 50 - 52 min, MeOH 100% back to 20% MeOH in H<sub>2</sub>O) yielded compound 3 (7 mg, R<sub>f</sub> = 0.72, 2 mg, respectively). Purification of F performed by the same gradient solvent system yielded compound 4 (4 mg, R<sub>f</sub> = 25...

[69x55]2 Iran J Pharm Res. 2022; 21(1):e126917.

[69x217]×

Ascentis® column (250 µm) equipped with a photodiode array detector (PDA) and an analytical HPLC system (Shimadzu Lc 10A, Tokyo, Japan) equipped with a photodiode array detector (PDA) and an analytical HPLC system (Shimadzu Lc 10A, Tokyo, Japan).
min) (Figure 1). Structure elucidation of the isolated compounds was carried out by spectrum data obtained from 1D-NMR (\textsuperscript{13}C and \textsuperscript{1}H-NMR) and mass (ESIMS). All data were in good agreement with those reported in the literature. The chemical structures of isolated compounds are shown in Figure 2.

4. Results and Discussion

This study evaluated the cytotoxic effects of crude extract and three fractions on MCF-7 cells by MTT assay. The results showed that the dichloromethane fraction had the most effective cytotoxic effect (Table 1).

| Total Extract | Petroleum Ether | Dichloromethane | n-Butanol |
|---------------|-----------------|-----------------|-----------|
| 987.98 ± 4.21 | 377.18 ± 1.36   | 297.17 ± 7.99   | 1094.85 ± 9.24 |

Nonpolar and semipolar extracts and fractions can be good candidates for cytotoxicity studies of the Artemisia genus. The polarity of the solvent plays a vital role in the extraction process. Dichloromethane is an organic solvent with a low polarity that can extract secondary metabolites with similar polarities, like terpenoids, coumarins, alkaloids, and polymethoxylated flavonoids. These types of phytochemical compounds can be responsible for cytotoxic effects on cancer cell lines (11, 12, 23, 24). Also, some mechanisms have been proposed for exerting cytotoxic effects of dichloromethane fractions and their isolated compounds, such as increasing the level of Bax protein, cleavage of PARP protein, and formation of DNA fragments (11, 14).

4.1. Spectroscopic Data of Isolated Compounds

Compound (1) 5-Hydroxy-3',4',6,7-tetramethoxyflavone (eupatilin 7-methyl ether): ESI-MS (m/z): 359.3 [M+H]\(^+\), yellow needle crystals, molecular formula C\(_{19}\)H\(_{18}\)O\(_7\); \textsuperscript{1}H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 12.75 (1H, s, 5-OH), 7.52 (1H, dd, J = 8.5, 2.2 Hz, H-6'), 7.34 (1H, d, J = 2.1 Hz, H-2'), 6.98 (1H, d, J = 8.6 Hz, H-5'), 6.59 (1H, brs, H-8), 6.55 (1H, brs, H-3), 3.99 (3H, s, 7-OMe), 3.98 (3H, s, 3'-OMe), 3.96 (3H, s, 4'-OMe), 3.93 (3H, s, 6-OMe). \textsuperscript{13}C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 182.78 (C-4), 164.14 (C-2), 158.91 (C-7), 153.40 (C-9), 153.24 (C-5), 152.47 (C-4'), 149.52 (C-3'), 132.84 (C-6), 123.09 (C-1'), 120.26 (C-6'), 111.32 (C-5'), 108.97 (C-2'), 106.33 (C-10), 90.77 (C-8), 61.05 (6-OMe), 56.50 (3'-OMe), 56.28 (4'-OMe), 56.04 (7-OMe), (25-29).

Compound (2) 5-hydroxy 3,3',4',6,7-pentamethoxyflavone (artemetin): ESI-MS (m/z): 389.3 [M+H]\(^+\), yellow crystals, molecular formula C\(_{20}\)H\(_{20}\)O\(_8\); \textsuperscript{1}H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 12.61 (1H, brs, 5-OH), 7.73 (1H, d, J = 8.5, 2.1, H-6'), 7.69 (1H, d, J = 2.1, H-2'), 6.99 (1H, d, J = 8.6, H-5'), 6.50 (1H, s, H-8), 3.97 (3H, s, 4'-OMe), 3.97 (3H, s, 3'-OMe), 3.96 (3H, s, 7-OMe), 3.92 (3H, s, 6-OMe), 3.87 (3H, s, 3-OMe). \textsuperscript{13}C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 178.89 (C-4), 158.78 (C-7), 155.94 (C-2), 152.8 (C-5), 152.33 (C-9), 151.46 (C-4'), 148.85 (C-3'), 138.81 (C-3), 132.35 (C-6), 122.09 (C-1'), 110.85 (C-5'), 106.63 (C-10), 90.38 (C-9), 60.9 (6-OMe), 60.22 (3-OMe), 56.50 (3'-OMe), 56.28 (4'-OMe), 56.04 (7-OMe), (30-32).

Compound (3) 6-methoxy-7-hydroxycoumarin (scopoletin): ESI-MS (m/z): 193.1 [M+H]\(^+\), beige needle crystal, molecular formula C\(_{10}\)H\(_8\)O\(_4\); \textsuperscript{1}H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.59 (1H, d, J = 9.5 Hz, H-4), 6.92 (1H, s, H-5), 6.85 (1H, s, H-8), 6.27 (1H, d, J = 9.5 Hz, H-3), 6.11 (1H, brs, 7-OH), 3.96 (3H, s, 6-OMe). \textsuperscript{13}C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 161.55 (C-2), 150.44 (C-7), 148.85 (C-4), 138.81 (C-3), 132.35 (C-6), 122.09 (C-1'), 110.85 (C-5'), 106.63 (C-10), 90.38 (C-9), 60.9 (6-OMe), 60.22 (3-OMe), 56.50 (3'-OMe), 56.28 (4'-OMe), (30-32).
Compound (4) methyl caffeate: ESI-MS (m/z): 193.1 [M-H], yellow needle crystals, molecular formula C_{10}H_{10}O_{4}; ^1H NMR (500 MHz, DMSO-d$_6$) $\delta$ 7.48 (1H, d, J = 16 Hz, H-7), 7.05 (1H, d, J = 2.2 Hz, H-2), 7.00 (1H, dd, J = 8.1, 2.0 Hz, H-6), 6.76 (1H, d, J = 8.1 Hz, H-5), 6.27 (1H, d, J = 15.9 Hz, H-8), 3.68 (3H, s, OCH$_3$). $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 167.08 (C-9), 148.48 (C-4), 145.62 (C-3), 145.19 (C-7), 125.50 (C-1), 121.4 (C-6), 115.73 (C-5), 114.84 (C-2), 113.71 (C-8), 51.24 (OCH$_3$) (35, 36).

Polymethoxyflavones (PMFs) belong to flavonoid compounds with two or more methoxy groups in the structure, imposing anti-inflammatory and anticancer pharmacological effects. Acetylation and hydroxylation of PMFs have been shown to reduce lipophilicity and improve their pharmacological effects. The in vitro and in vivo studies have revealed potent cytotoxic activity of these compounds in all three stages of cancer cells formation (initiation, promotion, and progression) (37). Eupatilin 7-methyl ether isolated from _A. argyi_ has shown a significant protective effect on contrast-induced nephrotoxicity by iodixanol in LLC-PK1 cells (38). Scopoletin belongs to coumarin compounds and has been found in _A. annua_ and _A. incisa_ (45, 46). Many pharmacological effects have been reported for scopoletin, such as antibacterial, antifungal, and antiproliferative (47). Scopoletin, isolated from the ethyl acetate extract of _A. argyi_, showed outstanding antiproliferative activity against CCRF-CEM leukemia cells with an IC$_{50}$ of 2.6 $\mu$M (48). Methyl caffeate has been reported from _A. integrifolia_, _A. argyi_, and _A. annua_ (49-51). In vitro studies have shown significant cytotoxic activity of cinnamic acid derivatives, like methyl caffeate, on different cancer cell lines (IC$_{50}$ ≤ 5 $\mu$g/mL) (52).

### 4.2. Conclusions

To the best of our knowledge, this is the first report on cytotoxic activity and phytochemical analysis of total extract and fractions of _A. haussknechtii_, which resulted in the isolation of compounds with cytotoxic potential on cancer cell lines. Various compounds isolated from this plant,
especially polymethoxylavones, can be considered for further studies.

Acknowledgments

The authors gratefully acknowledge the Research Affairs of Shahid Beheshti University of Medical Sciences for their financial support (Grant No. 99-19439).

References

1. Watson LE, Bates PL, Evans TM, Unwin MM, Estes JR. Molecular phylogeny of Subtribe Artemisinae (Asteraceae), including Artemisia and its allied and segregate genera. BMC Evol Biol. 2002;2:237. doi: 10.1186/1471-2148-2-37. [PubMed: 12350234]. [PubMed Central: PMC10036].

2. Taleghani A, Emami SA, Tayarani-Najaran Z. Artemisia: a promising plant for the treatment of cancer. Biogeochem Med Chem. 2020;28(1):e15180. doi: 10.1016/j.bmc.2019.15180. [PubMed: 31784499].

3. Hosseinzadeh L, Shokoohinia Y, Arab M, Allahyari E, Mojarrah M. Cytotoxic and Apoptogenic Sesquiterpenoids from the Petroleum Ether Extract of Artemisia aucheri Aerial Parts. Iran J Pharm Res. 2019;18(1):391–9. doi: 10.18861/PM.18089171. [PubMed Central: PMC6847405].

4. Hajdu Z, Hohmann J, Furgó, P. Mitochondrial cytochrome c release in HeLa cells. J Cell Physiol. 2010;226(3):692–7. doi: 10.1002/jcp.22496. [PubMed: 20595670].

5. Hong L, Ying SH. Ethanol extract and isolated constituents from Artemisia dracunculus inhibits esophageal squamous cell carcinoma and induce apoptotic cell death. Drug Res (Stuttg). 2019;69(2):100–6. doi: 10.1055/s-0039-1672647. [PubMed: 25076244].

6. Yuan H, Lu X, Ma Q, Li D, Xu G, Piao G. Flavonoids from Artemisia echinata L. J Med Plant Res. 2016;10(4):e1692-7. doi: 10.5555/2016.10.4.e1692. [PubMed: 25295670].

7. Nikbakht MR, Sharifi S, Emami SA, Khodaei L. Chemical composition and antiproliferative activity of Artemisia persica Boiss. and Artemisia turcomanica Gand. essential oils. Res Pharm Sci. 2014;9(2):55–63. doi: 10.5502/resp.2014.09.02. [PubMed: 25567784]. [PubMed Central: PMC4129293].

8. LI, Y, LI MY, WANG L, LIANG ZH, LI WY, LI H. Induction of apoptosis of cultured hepatocarcinoma cell by essential oil of Artemisia annulata L. Sichuan Da Xue Xue Bao Yi Xue Ban. 2004;35(3):337–9. Chinese. [PubMed: 15818292].

9. Zhang LM, LV XW, SHAO LX, MA YF, CHENG WZ, GAO HT. [Essential oil from Artemisia lavandulaefolia induces apoptosis and necrosis of Hela cells]. Zhong Yiu Cai. 2013;36(12):1988–92. Chinese. [PubMed: 25090687].

10. Saleh AM, Almadi A, Rizvi SA, Nasr A, Alaskar AS, Williams JD. In vitro cytotoxicity of Artemisia vulgaris L. essential oil is mediated by a mitochondria-dependent apoptosis in HL-60 leukemic cell line. BMC Complement Altern Med. 2014;14:226. doi: 10.1186/1472-6882-14-226. [PubMed: 25002129]. [PubMed Central: PMC4227289].

11. Tayarani-Najaran Z, Makki PS, Alamholodia NS, Mojarrah M, Emami SA. Cytotoxic and apoptotic effects of different extracts of Artemisia biennis Willd. on K562 and HL-60 cell lines. Iran J Basic Med Sci. 2017;20(2):166–71. doi: 10.22203/ijbms.2017.8242. [PubMed: 28293393].

12. Tayarani-Najaran Z, Hajian Z, Mojarrah M, Emami SA. Cytotoxic and apoptotic effects of extracts of Artemisia cinformis Krusch. and Popov ex Poljak on K562 and HL-60 cell lines. Asian Pac J Cancer Prev. 2014;15(7):7055–9. doi: 10.7345/ajpcr.2014.15.7.7055. [PubMed: 25227790].
29. Awad BM, Habbib ES, Ibrahim AK, Wanas AS, Radwan MM, Helal MA, et al. Cytotoxic activity evaluation and molecular docking study of phenolic derivatives from Achillea fragrantissima (Forssk.) growing in Egypt. *Med Chem Res*. 2017;26(9):2065-73. doi: 10.1007/s10044-017-1918-6.

30. Huo C, Li Y, Zhang M, Wang Y, Zhang Q, Qin F, et al. Cytotoxic flavonoids from the flowers of Achillea millefolium. *Chem Nat Compd*. 2013;48(6):3958-62. doi: 10.1007/s10600-013-0438-y.

31. Lan J, Li J, Zhu X, Sun ZL, He JM, Zhol M, et al. Flavonoids from Artemisia rupestris and their synergistic antibacterial effects on drug-resistant Staphylococcus aureus. *Nat Prod Res*. 2021;35(11):1881-6. doi: 10.1080/14786419.2019.1639182. [PubMed: 31303068].

32. Qadir M, Dangroo NA, Agnihotri VK, Shah WA. Isolation, characterisation, antifungal activity and validated UPLC/MS/MS method for quantification of novel compound from Artemisia tournefortiana Reichb. *Nat Prod Res*. 2021;35(9):1381-8. doi: 10.1080/14786419.2021.1915310.

33. Kamau RW, Juma BF, Baraza LD. Antimicrobial compounds from root, stem bark and seeds of Mella volkensii. *Nat Prod Res*. 2016;30(7):1984-7. doi: 10.1080/14786419.2015.1010104. [PubMed: 26517430].

34. Gu X, Bai B, Chen Y, Wang M, Dong Y, Yuan C, et al. Chemical Constituents from the Tubers of Kosteletzkya virginica. *Chem Nat Compd*. 2016;52(2):336-8. doi: 10.1007/s10600-016-1644-4.

35. Lima TC, Ferreira AR, Silva DF, Lima EO, de Sousa DP. Antifungal activity of cinnamic acid and benzoic acid esters against Candida albicans strains. *Nat Prod Res*. 2018;32(5):572-5. doi: 10.1080/14786419.2017.1317776. [PubMed: 28421912].

36. Tung Y, Chou Y, Hung W, Cheng A, Yu R, Ho C, et al. Induced Cytotoxicity by Iodixanol in LLC-PK1 Cells. *Int J Mol Sci*. 2018;19(5). doi: 10.3390/ijms19051387. [PubMed: 29735908]. [PubMed Central: PMC5983776].

37. Livingstone J, Chou Y, Hung W, Cheng A, Yu R, Ho C, et al. Prevention and Treatment. *Curr Pharmacol Rep*. 2018;4(2):98-113. doi: 10.1007/s40495-019-0070-z.

38. Lee D, Kim CE, Park SY, Kim KO, Hiep NT, Lee D, et al. Protective Effect of Artemisia argyi and Its Flavonoid Constituents against Contrast-Induced Cytotoxicity by Iodixanol in LLC-PK1 Cells. *Int J Mol Sci*. 2018;19(5). doi: 10.3390/ijms19051387. [PubMed: 29735908]. [PubMed Central: PMC5983776].

39. Kikhanova ZS, Iskakova ZB, Dzhalmakhanbetova RI, Seilkhanov TM, Ross SA, Suleimen EM. Constituents of Artemisia austriaca and their Biological Activity. *Chem Nat Compd*. 2013;49(5):5967-8. doi: 10.1007/s10517-013-0796-5.

40. Seo JM, Kang HM, Son KH, Kim JH, Lee CW, Kim HM, et al. Antitumor activity of flavones isolated from Artemisia argyi. *Planta Med*. 2001;69(3):218-22. doi: 10.1055/s-2003-18486. [PubMed: 12677524].

41. Tang HQ, Hu J, Yang L, Tan RX. Terpenoids and flavonoids from Artemisia species. *Planta Med*. 2000;66(4):391-4. doi: 10.1055/s-2000-8538. [PubMed: 10865468].

42. Xiao J, Jiao F, Zhao X, Zhong W, Duan L, Wang L, et al. Rutepenic acids H and I, two new sesquiterpenes from the flowers of Artemisia rupestris L. *Phytochem Lett*. 2018;27:78-81. doi: 10.1016/j.phytol.2018.06.008.

43. Ortel R, Prado S, Regaldo EL, Valeriote FA, Media J, Mendolia J, et al. Furfuran lignans and a flavone from Artemisia gorgonum Webb and their in vitro activity against Plasmodium falciparum. *J Ethnopharmacol*. 2011;138(2):637-40. doi: 10.1016/j.jep.2011.09.039. [PubMed: 21982788].

44. Awad HM, Abd-Alla HI, Mahmoud KH, El-Toumy SA. In vitro anti-nitrosative, antioxidant, and cytotoxicity activities of plant flavonoids: a comparative study. *Med Chem Res*. 2014;23(7):3298-307. doi: 10.1007/s10044-014-0915-2.

45. Wang MJ, Wang JL, Wang D, Shi ZC, Li J, Zhao M, et al. Study on chemical constituents of Artemisia incisa Pamp (Asteraceae). *J Ethnopharmacol*. 2019;234:323-8. doi: 10.1016/j.jep.2019.02.022. [PubMed: 30822508].

46. Rashid MI, Alamzeh M, Ali S, Shah ZA, Naz I, Khan AA, et al. A new irregular monoterpen acetate along with eight known compounds with antifungal potential from the aerial parts of Artemisia incisa Pamp (Asteraceae). *Nat Prod Res*. 2017;31(4):428-35. doi: 10.1080/14786419.2016.1185718. [PubMed: 27878015].

47. Firmansyah A, Wininggihw W, Manobi DY. Review of Scopoletin: Isolation, Analysis Process, and Pharmacological Activity. *Biointerface Res Appl Chem*. 2021;11(4):12006-19. doi: 10.33263/briac114.1200612019.

48. Adams M, Effert T, Bauer R. Activity-guided isolation of scopoletin and isoscopoletin, the inhibitory active principles towards CCRF-CEM leukaemia cells and multi-drug resistant CEM/ADR5000 cells, from Artemisia argyi. *Planta Med*. 2006;72(9):862-4. doi: 10.1055/s-2006-947165. [PubMed: 16881093].

49. Wang MJ, Wang JL, Wang D, Shi ZC, Li J, Zhao M, et al. Study on chemical constituents of Artemisia integrifolia (II). *Zhong Cao Yao*. 2019;50:5411-8.

50. Kim KO, Lee D, Hiep NT, Song JH, Lee HJ, Lee D, et al. Protective Effect of Phenolic Compounds Isolated from Mugwort (Artemisia argyi) against Contrast-Induced Apoptosis in Kidney Epithelium Cell LLC-PK1 Cells. *J Plant Biochem Biotechnol*. 2019;24(3). doi: 10.1390/molecules24010895. [PubMed: 30620854]. [PubMed Central: PMC6377078].

51. Wang Q, Hou GM, Li DY, Li ZL. A new coumarin glycoside isolated from Artemisia annua. *Zhong Cao Yao*. 2018;29(9):8537-8. [PubMed: 10865468].

52. Nam NH, You YJ, Kim Y, Hong DH, Kim HM, Ahn BZ. Syntheses of certain 3aryl-2-propenoates and evaluation of their cytotoxicity. *Bioorg Med Chem Lett*. 2001;11(9):3173-6. doi: 10.1016/j.bmcl.2001.06.052. [PubMed: 11354370].