کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی

کارگاه آنلاین
کاربرد نرم افزار SPSS در پژوهش

کارگاه آنلاین
اصول تجزیه و تحلیل قراردادها

کارگاه آنلاین
پروپوزال نویسی
Investigation of Cytotoxic Activity in Four Stachys Species from Iran

Mahnaz Khanavi\textsuperscript{a}, Azadeh Manayi\textsuperscript{a}, Mahnaz Lotfi\textsuperscript{b}, Rofeyde Abbasi\textsuperscript{b}, Maryam Majdzadeh\textsuperscript{a} and Seyed Nasser Ostad\textsuperscript{c}*

\textsuperscript{a}Department of Pharmacognosy, Faculty of Pharmacy and Traditional Iranian Medicines and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran. \textsuperscript{b}Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran. \textsuperscript{c}Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Abstract

The aerial parts of \textit{Stachys laxa} Boiss. and Buhse. from Siah-bishe in Mazandaran province, \textit{Stachys trinervis} Aitch. and Hemsl. from Karaj in Alborz province, \textit{Stachys subaphylla} Rech. F. and \textit{Stachys turcomanica} Trautv. from Golestan province have been collected in May 2008. Total extracts were obtained through MeOH/H\textsubscript{2}O (80/20) and then partitioned between CHCl\textsubscript{3}, EtOAc and MeOH. These fractions and total extracts have been investigated for in-vitro cytotoxic activity against the colon carcinoma (HT-29), colorectal adenocarcinoma (Caco-2), breast ductal carcinoma (T47D) and Swiss mouse embryo fibroblast (NIH 3T3) cell lines using MTT assay (3-(4,5-di methyl thiazol-2-yl)-2,5-di phenyltetrazolium bromide). At each cell line, doses of 3.125, 6.25, 12.5, 25, 100, 200, 400 and 800 µg/mL in 1% (v/v) DMSO of all samples were tested. Ethyl acetate and chloroform fractions of \textit{Stachys laxa} against proliferation of T47D and HT-29 cell lines and chloroform fraction of \textit{Stachys subaphylla} and \textit{Stachys subaphylla} ethyl acetate fraction toward T47D cell line exhibited highest cytotoxic activity (IC\textsubscript{50} < 50 µg/mL). Ethyl acetate and chloroform fractions of \textit{Stachys turcomanica} against HT-29 cell line, except methanol fraction of \textit{Stachys subaphylla}, the other extracts on T47D cell line, represented moderate cytotoxic activity (IC\textsubscript{50} < 70 µg/mL). All fractions of \textit{S. trinervis} demonstrated no effective cytotoxic activity. IC\textsubscript{50} values confirmed that the growth and proliferation of HT-29 and T47D cells were most affected by chloroform and ethyl acetate fractions of \textit{Stachys laxa} and \textit{Stachys turcomanica} due to their nonpolar compounds.

Keywords: Cytotoxic activity; \textit{Stachys laxa} Boiss. and Buhse.; \textit{Stachys turcomanica} Trautv.; \textit{Stachys subaphylla} Rech. F.; \textit{Stachys trinervis} Aitch. and Hemsl.; MTT assay.

Introduction

The genus \textit{Stachys} belongs to the plant family of Lamiaceae. The most species of this genus has been previously analyzed in numerous studies concerning their chemical composition, pharmacological properties and therapeutic uses. This family is well represented in the flora of Iran, at least with 200-300 species in the world (1) and 34 species in Iran (2). Phytochemical investigation of some \textit{Stachys} species has demonstrated phenolic acids, tannins (3, 4), flavonoids (5) and phenyl ethanoid glycosides (6, 7). There are some reports about pharmacological activities of this genus including anticancer (8,
were collected in May 2008. The plants have been identified and deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

**Extraction**

Freshly collected aerial parts of four species of *Stachys* were cleaned and shade dried. These parts were coarse powdered in a hand mill and stored at room temperature. Two hundred grams of powdered plants were extracted through percolation method with 80% aq. MeOH three times at room temperature. The extract was evaporated using rotary evaporator and consequently partitioned between CHCl$_3$, EtOAc and MeOH. Each fraction evaporated with rotary evaporator and has been stored at refrigerator for the investigation of cytotoxic activity.

**Cytotoxicity assay**

The colon carcinoma (HT-29), colorectal adenocarcinoma (Caco-2) and ductal carcinoma (T47D) cell lines were mentioned as exponentially growing cultures in RPMI 1640 cell culture medium (PAA, Germany), supplemented with 10% fetal bovine serum (FBS: Gibco, USA), for HT-29 cells and 15% FBS for Caco-2 and T47D cells. The Swiss mouse embryo fibroblast (NIH 3T3) cell line was kept in Dulbecco’s modified Eagle’s medium (DMEM; PAA, Germany) supplemented with 10% FBS. 100 IU/mL penicillin and 100 µg/mL streptomycin (Roche, Germany) were added to the media. All the cell lines were cultured at 37°C in air /carbon dioxide (95:5) atmosphere.

Cytotoxic activity was measured using modified MTT assay (31). 1×10$^4$ cells/well were plated in 96-well plates (Nunc, Denmark) and incubated for 24 h before the addition of drugs. After 96 h of incubation in Caco$_2$ cells and 48 h of incubation in HT-29, NIH/3T3 and T47D cells, 20 µL of MTT (Merck, Germany) reagent (5 mg/mL) in phosphate buffered saline (PBS) was added to each well. The plates were incubated at 37°C for 4 h. The medium was discharged and the formazan blue, which had been formed in the cells, were dissolved with 100 µL dimethyl sulfoxide (DMSO). After the incubation at 37°C for 10 min, absorbance at 570 nm at the dissolved solutions was detected using a micro plate reader.

**Experimental**

**Plant material**

The aerial parts of *S. laxa* Boiss. and Buhse., from Siah-bishe in Mazandaran province, *S. trinervis* Aitch. and Hemsl from Karaj in Alborz province, and *S. subaphylla* Rech. F. and *S. turcomanica* Trautv. from Golestan province were collected in May 2008. The plants have been identified and deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
Investigation of Cytotoxic Activity in Four Stachys Species from Iran

The cell viability in MTT assay was calculated as the percentage of control value. Methotrexate was used as the positive control. Cytotoxicity was expressed as the concentration of extract inhibiting cell growth with 50% (IC\textsubscript{50} ± SD). All tests and analysis were run in triplicate.

Statistical analysis

IC\textsubscript{50} (the median growth inhibitory concentration) values were calculated from the IC\textsubscript{50} of dose-response curve in the sigma plot 11 software. Data representative of three independent experiments with similar results were presented as mean ± SD.

Result

The effects of these plant extracts on the proliferative response of the HT-29, Caco-2 and T47D cell lines have been analyzed by treating the cells with different concentrations of the extracts and significant decrease in cell lines proliferation were observed. IC\textsubscript{50} ± SD are reported in Table 1. The chloroform and ethyl acetate fractions of \textit{S. laxa} Boiss. showed high cytotoxicity on T47D, HT-29 (IC\textsubscript{50} < 50 µg/mL).

Discussion

Among all the samples, nonpolar (chloroform and ethyl acetate fractions) fractions of \textit{S. laxa} exhibited greatest cytotoxicities on T47D and HT-29 cell lines compared with polar fraction and total extract. According to the data, the

Table 1. Cytotoxic activity of total extract and fractions of four species of \textit{Stachys}.

| Sample         | Cell Lines* (MTT assay) |
|----------------|-------------------------|
|                | HT-29       | Caco-2    | T47D      | NIH/ 3T3   |
| \textit{Stachys laxa} |           |           |           |           |
| Total extract  | 421.97 ± 8.71 | > 1000    | 239.78 ± 16.92 | 508.77 ± 45.56 |
| Methanol fr.   | 265.83 ± 46.52 | > 1000    | 254.1 ± 7.45  | 405.7 ± 74.18 |
| Ethyl acetate fr. | 134.004 ± 1.764 | 116.53 ± 18.23 | 18.079 ± 2.248 | 31.452 ± 1.554 |
| Chloroform fr. | 27.007 ± 2.096  | 101.65 ± 12.4  | 21.106 ± 2.491 | 41.294 ± 8.391 |
| \textit{Stachys subaphylla} |           |           |           |           |
| Total extract  | > 1000      | > 1000    | 60.15 ± 3.72  | 771.26 ± 164.67 |
| Methanol fr.   | > 1000      | > 1000    | 711.26 ± 197.38 | > 1000      |
| Ethyl acetate fr. | -           | 116.52 ± 2.78  | 51.05 ± 8.89  | -           |
| Chloroform fr. | 234.86 ± 11.28 | 183.85 ± 8.87  | 43.411 ± 9.99  | 74.27 ± 2.34 |
| \textit{Stachys trinervis} |           |           |           |           |
| Total extract  | > 1000      | > 1000    | 358.1 ± 14.14 | > 1000      |
| Methanol fr.   | > 1000      | > 1000    | 630.96 ± 29.99 | 649.23 ± 17.91 |
| Ethyl acetate fr. | 241.66 ± 14.71 | 338.22 ± 1.02  | 128.35 ± 6.65  | 110.05 ± 5.56 |
| Chloroform fr. | > 1000      | > 1000    | 383 ± 4.01    | 674.84 ± 67.37 |
| \textit{Stachys turcomanica} |           |           |           |           |
| Total extract  | 219.58 ± 14.21 | > 1000    | 103.67 ± 12.43 | 308.7 ± 1.34 |
| Methanol fr.   | 693.57 ± 56.91 | > 1000    | 708.60 ± 25.8  | 802.58 ± 26.84 |
| Ethyl acetate fr. | 66.10 ± 5.43  | 87.08 ± 8.9  | 30.14 ± 6.65   | 50.45 ± 3.45 |
| Chloroform fr. | 66.84 ± 7.92  | 187.89 ± 11.72 | 51.38 ± 9.49  | 58.22 ± 4.06 |
| Methotrexate   | 0.23 ± 0.02  | 0.32 ± 0.04  | 0.16 ± 0.09   | 0.24 ± 0.013 |

*Results are expressed as IC\textsubscript{50} values (µg/mL). Key to cell Lines employed: HT-29 and Caco-2 (colon Adenocarcinoma), T47D (breast carcinoma), NIH 3T3 (Swiss embryo fibroblast.

(Anthos, Austria). The cell viability in MTT assay was calculated as the percentage of control value.
cytotoxic activity of chloroform and ethyl acetate fractions on HT-29 and T47D cell lines were much stronger than that of Caco-2. It indicated that chloroform and ethyl acetate fractions of S. laxa had potential cytotoxic selectivity on T47D cell line. There was a report about the antioxidant and total phenol content of some Stachys spp. The research implied that total phenol content and FRAP value of methanolic extracts are in this order: S. laxa > S. turcomanica > S. subaphylla > S. trinervis (33). Except S. subaphylla total extract against T47D cell line, the other methanolic extracts have indicated the same order of cytotoxic activity on T47D and HT-29. Higher cytotoxic activity of nonpolar fraction of S. laxa and S. turcomanica may be due to the high content of germacrene D in their essential oil, same as the Stachys cretica ssp. (34, 35), but main components of S. subaphylla and S. trinervis essential oils were identified as monoterpenic hydrocarbons. In comparison with another fraction, methanolic and total fractions of all samples demonstrated slightly cytotoxic effect on cell line tested. The real IC₅₀ values of fractions of four species Stachys may be considerably lower than the positive control (Methotrexate) since its pharmacological active compounds are not pure and further researches are needed for defining potential component as cytotoxic natural medicines.

References

(1) Rechinger KH and Hedge IC. Flora Iranica. Akademisch Druck-und Verlagsanstalt, Graz, Austria (1982) 150: 360-361.
(2) Mozaffarian V. a Dictionary of Iranian Plant Names. Farhang Moaser, Tehran (1996) 522.
(3) Vundac VB, Brantner AH and Plazibat M. Content of phenolic constituents and antioxidant activity of some Stachys taxa. Food Chem. (2007) 104: 1277-81.
(4) Vundac VB, Males Z, Plazibat M, Golja P and Cetina-Cizmek BC. HPTLC determination of flavonoids and phenolic acids in some Croatian Stachys taxa. J. Planar Chromatogr. Mod. TLC (2005) 18: 269-73.
(5) El-Ansari MA, Nawwar MA and Saleh NAM. Stachysetin, a diapigenine-7-glucoside-p-p-dihydroxytruxinate from Stachys aegyptiaca. Phytochem. (1995) 40: 1543-48.
(6) Miyase T, Yamamoto R and Ueno A. Phenyl ethanolid glycosides from Stachys officinalis. Phytochem. (1996) 43: 475-79.
(7) Nishimura H, Sasaki H, Inagaki N, Chin M and Mitsuhashi H. Nine phenethyl alcohol glycosides from Stachys setiboldii. Phytochem. (1991) 30: 659-69.
(8) Amirghofran Z, Bahmani M, Azadmehr A and Javidnia K. Anticancer effects of various Iranian native medicinal plants on human tumor cell lines. Neoplasma (2006) 53: 428-33.
(9) Amirghofran Z, Bahmani M, Azadmehr A and Javidnia K. Immunomodulatory and apoptotic effects of Stachys obtusiflora on proliferative lymphocytes. Med. Sci. Monit. (2007) 13: 145-50.
(10) Statamis G, Kyria opoulos P, Golegov S, Basayiannis A, Skaltsas S and Skaltsa H. In-vitro anti-Helicobacter pylori activity of Greek herbal medicines. J. Ethnopharmacol. (2003) 88: 175-9.
(11) Grujic-Jovanovic S, Skaltsa HD, Marin P and Sokovic M. Composition and antibacterial activity of the essential oil of six Stachys species from Serbia. Flav. Fragr. J. (2004) 19: 139-44.
(12) Sonboli A, Salehi P and Nejad Ebrahimi S. Essential oil composition and antibacterial activity of the leaves of Stachys scheidegleevii from Iran. Chem. Nat. Compd. (2005) 41: 171-4.
(13) Digrag M, Hakki Alma M and Ilcin A. Antibacterial and antifungal activities of Turkish medicinal plants. Pharm. Biol. (2001) 39: 346-50.
(14) Aydin A, Sener B, Cakici I, Turan NN and Erdemoglu N. Antioxidant activities of some Lamiaceae plant extracts. Phytother. Res. (2006) 20: 91-3.
(15) Kukik J, Petrovic S and Niketic M. Antioxidant activity of four endemic Stachys taxa. Biol. Pharm. Bull. (2006) 29: 725-9.
(16) Matkowski A and Piotrowska M. Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. Fitoterapia (2006) 77: 346-53.
(17) Khanavi M, Sharifzadeh M, Hadijakhooodi A and Shafiee A. Phytochemical investigation and anti-inflammatory activity of aerial parts of Stachys byzantina C. Koch. J. Ethnopharmacol. (2005) 97: 463-8.
(18) Khanavi M, Sharifzadeh M, Hadijakhooodi A and Shafiee A. Anti-inflammatory activity of aerial part of Stachys byzantina C. Koch. Iranian J. Pharm. Res. (2004) Supp. 2: 55-56.
(19) Kukic J, Dobric S and Petrovic S. Influence of some Stachys taxa on carrageenan-induced paw edema in rats. Pharm. Biol. (2007) 45: 560-3.
(20) Maleki N, Garjani A, Nazemiyah H, Nifouroshah N, Efekhar Sadat AT, Aalamel Z and Hasannia N. potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of Stachys inflata on rats. J. Ethnopharmacol. (2001) 75: 213-8.
(21) Sharifzadeh M, Sharifzadeh K, Khanavi M, Hadijakhooodi A and Shafiee A. Anti-inflammatory activity of aerial parts of Stachys setifera and Stachys persica. Int. J. Pharmacol. (2005) 1: 132-7.
(22) Skaltsa HD, Bermejo P, Lazar M, Silvan AM, Skaltsounis AL, Sanz A and Abad MJ. Inhibition of prostaglandin E₂ and leukotriene C₅ in mouse peritoneal macrophages and thromboxan B₂ production in human
platelets by flavonoids from Stachys chrysanthana and Stachys candida. Biol. Pharm. Bull. (2000) 23: 47-53.

(23) Hayashi K, Nagamatsu T, Ito M, Hattori T and Suzuki Y. Acotoside, a component of Stachys sieboldii MIQ, may be a promising antinephritic agent. Effects of acotoside on crescentic-type anti-GBM nephritis in rats. Jpn. J. Pharmacol. (1994) 65: 143-51.

(24) Rabbani M, Sajjadi SE and Zarei HR. Anxiolytic effects of Stachys lavandulifolia Vahl on the elevated plus-maze model of anxiety in mice. J. Ethnopharmacol. (2003) 89: 271-6.

(25) Gruenwald J, Brendler T and Jaenicke C. PDR for Herbal Medicines. 2nd ed., Medical Economics Company, New Jersey (2000) 832.

(26) Şerbetçi T, Demirci B, Güzele ÇB, Kültür Ş, Ergüven M and Başer KHC. Essential oil composition, antimicrobial and cytotoxic activities of two endemic Stachys cretica subspecies (Lamiaceae) from Turkey. Nat. Prod. Commun. (2010) 5: 1369-74.

(27) Firouznia A, Rustaiyan A, Masoudi S, Rahimizade M, Bigdeli M and Tabatabaei-Anaraki M. Volatile constituents of Salvia limbata, Stachys turcomanica, Scutellaria litvinowii and Hymenocramer elegans four Lamiaceae herbs from Iran. J. Essent. Oil-Bearing Plants (2009) 12: 482-9.

(28) Khanavi M, Farahankia B, Janbakhsh S, Sheirani S, Hoseini-Sajjadi SM, Salahi-Oliaee MH, Ajani Y and Hadjiakhoondi A. Comparison of the essential oil composition of Stachys trinervis Aitch. & Hemsl. and Stachys subaphylla Rech. F. J. Essent. Oil-Bearing Plants (2008) 11: 406-12.

(29) Sajjadi SE and Mehrregan I. Composition of the essential oil of Stachys laxa Boiss. & Buhse. Iranian J. Pharm. Res. (2003) 8: 57-58.

(30) Háznga-Radnai E, Réthy B, Czigle Sz, Zupkó I, Wéber E, Martinek T, Falkay Gy and Máthé I. Cytotoxic activities of Stachys species. Fitoterapia (2008) 79: 595-7.

(31) Newman DJ and Cragg GM. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. (2007) 70: 461-77.

(32) Rahman A, Choudhary MI and Thomsen WJ. Bioassay Techniques for Drug Development. Taylor and Francis Group, Netherlandes (2001) 34-35.

(33) Khanavi M, Hajimahmoodi M, Cheraghi-Niroomand M, Kargar Z, Ajani Y, Hadjiakhoondi A and Oveisi MR. Comparison of the antioxidant activity and total phenolic contents in some Stachys species. Afr. J. Biotechnol. (2009) 8: 1143-7.

(34) Kuźma Ł, Kalambo D, Różalski M, Różalska B, Więckowska-Szakiel M, Krajewska U and Wysokińska H. Chemical composition and biological activities of essential oil from Salvia sclarea plants regenerated in-vitro. Molecules (2009) 14: 1438-47.

(35) Richmond JD, Agius BR, Wright BS, Haber WA, Moriarity DM and Setzer WN. Essential oil compositions and cytotoxic activities of Dendropanax capillaris, Oreopanax nubigenus, and Schefflera rodriqueziana from Monteverde, Costa Rica. Nat. Prod. Commun. (2007) 4: 271-4.

This article is available online at http://www.ijpr.ir
