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Full Length Research Paper

Biological activity of the n-butanolic extract of *Stachys pilifera*

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The n-butanolic extract of *Stachys pilifera* growing in Iran have been screened for antimicrobial, antioxidant and antitumor activity. The antimicrobial capacity of this extract was evaluated against Gram-positive and Gram-negative bacteria, and fungi. The extract was showed mildly significant activity against these microorganisms. The antioxidant activities were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. IC_{50} values in the DPPH assay 2.8 mg/mL. This plant showed the high antioxidant activities. DPPH assay results showed good correlations with the total phenolic contents of the plants, measured by the Folin-Ciocalteau assay: ($r^2 = 0.871$, $p < 0.0001$). The results of antitumor activity were showed that the extract exhibit antitumor properties. It was showed enhanced inhibitory activity compared to control compounds. It has also been proposed that concentration plays a vital role in increasing the degree of inhabitation.

Key words: Antioxidant, antitumor, 2,2-diphenyl-1-picrylhydrazyl (DPPH), total phenol, antimicrobial.

INTRODUCTION

Since ancient times the crude herbal extracts of aromatic plants have been in use for different purposes, such as food, drugs and perfumery. These extracts have recently gained popularity and scientific interest because of their uses in the food, drug, and perfumery industries (Heath, 1981). Scientists have been interested in bio-logically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that they have built against antibiotics (Essawi and Srour, 2000). Plant products could be useful in preserving food storages from contamination (Bandoniene et al., 2002), whereas the synthetic antioxidants that have been used previously are now toxicologically suspect (Grice, 1986; Wichi, 1988).

Stachys is a Greek word, meaning “ear of corn,” or “spike,” and refers to the arrangement of flowers on the stem. This genus with about 300 species of annuals and distributed extensively in the tropical and subtropical countries, except for Australia and New Zealand (Brown, 1995). 34 species of this genus are found in Iran of which 13 species are endemic (Ghahreman, 1995; Rechinger, 1982; Zargari, 1992). Phytochemical studies on *Stachys* species has confirmed the presence of phenolic compounds and glycosides (Nazemieh at al., 2006; Yin et al., 2006), terpenoids and steroids (Ross and Zinchenko, 1975; Yamamoto et al., 1994), diterpenes (Piozzi et al., 1980) and flavonoids and phenolic acids (Vundac et al., 2005) in these plants. Also, biological studies have demonstrated considerable antibacterial, antiinflammatory, antitoxic, antinephritic, antihepatitis and antianoxia effects for some *Stachys* species (Hayashi et al., 1994a, b; Savchenko and Khvorostinka,1978; Skaltsa et al., 1999; Yamahara et al.,1990; Zinchenko et al., 1981).

Stachys pilifera is one of the endemic species of Iran and its aerial parts are orally used as herbal tea in the treatment of various infections, asthmatic, rheumatic and other inflammatory disorders (Zargari, 1992). Fresh flowering tops of the plant are also frequently used in

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jams and pickles as flavoring agents (Ghahreman, 1995).

To the best of our knowledge, reports on the antimicrobial profiles of *S. pilifera* are scant and there is no report on its antioxidant and antitumor potential in the literature. Thus, the present research reports, (i) *in vitro* antioxidant activity profiles of the extract of this plant using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and total phenolic compounds content of the plant extracts as gallic acid equivalents, (ii) antimicrobial potentials of the plant extract using two complementary methods, namely disc diffusion method and the minimal inhibitory concentration (MIC) (iii) *in vitro* cytotoxic activity of *S. pilifera* extract against colon carcinoma (TH-29) and breast ductal carcinorma (T47D) cell lines.

MATERIALS AND METHODS

Reagents and chemicals

Trolox (water soluble equivalent of vitamin E) and quercetin were from Acros Organics (Geel, http://www.acros.com). Acetic acid glacial, dimethyl sulphoxide, ferrous sulphate heptahydrate, ferric chloride, Folin–Ciocalteau reagent, hexane, methanol, sodium acetate, sodium carbonate and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Merck (Darmstadt, http://www.merck.de). Galic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and hydrochloric acid 32% were obtained from Sigma–Aldrich (St. Louis, http://www.sigma-aldrich.com).ase submit your manuscript electronically for review as e-mail attachments.

Sample

The plant materials were collected in June 2011 from sepidan road (Fras Province, Iran). Voucher specimens have been deposited at the herbarium of the Research Institute of Forests and Rangelands by Dr. Mozaffarian, Tehran, Iran in Voucher No. AR-143-B. Aerial parts of plants were air-dried at room temperature (25°C) in the shade.

Extraction

Butanolic extracts of the plant were prepared as follows: 7.5 g dry plant, after being defatted with light petroleum, was macerated in 200 ml n-Butanol for 2 days with one change of solvent after 1 day. The extract was filtered and then concentrated in a rotary vapor in less than 10 min. powders were weighed to calculate the yield, and kept at -20°C until used. Shortly before each experiment, the powder was dissolved in methanol at the desired concentration.

Statistical analysis

Regression analyses were performed by SigmaPlot 2002 for Windows version 8.0.

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial activities of the *S. pilifera* were determined by using the agar dilution procedure outlined by the disc-diffusion method (Bauer et al., 1966) and determining the minimal inhibitory concentration (MIC). Plant extract and controls were tested in duplicate and the experiments were repeated four times. Minimal inhibitory concentrations for extract was investigated against standard bacterial strains *Bacillus pumilus* (PTCC 1319), *Escherichia coli* (PTCC 1533), *Kocuria varians* (PTCC 1484), *Listeria monocytogenes* (PTCC 1298) and *Salmonella typhi* (PTCC 1609). The fungal strains that were used in this study were *Aspergillus niger* (PTCC 5154), *Aspergillus flavus* (PTCC 5006) and *Candida glabrata* (PTCC 5297). All microorganisms were obtained from the Persian type culture collection (PTCC), Tehran, Iran. Microorganism strains were prepared to turbidity equivalent to McFarland Standard No. 0.5 (Janssen et al., 1987). The suspen-sions were then spread on a test plate of Muller–Hinton agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India). Sterile discs were impregnated with 2 mg of the plant extract and placed on the surface of the test plate. All the inoculated plates were incubated at 35°C and the results were evaluated after 16–20 h of incubation for bacteria and 48 h for fungi. The lowest concentration of the com-pounds that prevented visible growth was considered to be the minimal inhibitory concentration (MIC). In the disc-diffusion method, Plates were incubated at 37°C for 24 h, at which the diameters of inhibition zones were measured in mm. Each assay was repeated three times, and the means were calculated. Positive control discs were included gentamicin, ampicillin and ketoconazole for Gram-negative bacteria, Gram-positive bacteria and fungi, respectively. The extract of *S.pilifera* was showed mildly significant activity against these microorganisms (Table 1).

Antioxidant activity

The growing interest in the substitution of synthetic food antioxidants with natural ones has fostered research on plant sources and screening of raw materials to identify new antioxidants. Interest in oxidation reactions is not confined to the food industry, as antioxidants are widely needed to prevent deterioration of other perishable goods, such as cosmetics, pharmaceuticals and plastics. In addition, other biological properties such as anticarcinogenicity, antimutagenicity, antiallergenicity and antiaging activity have been reported for natural and synthetic antioxidants (Moure et al., 2001). Several methods have been used to determine antioxidant activity of plants. Our present study therefore involved two various established methods to evaluate antioxidative activity of this extract, namely, DPPH radical-scavenging activity and total phenol antioxidant capacity. Antioxidant activities of the plant extracts are reported in (Table 2). Total phenolic contents of the tested plants showed good correlations with the results of DPPH methods (r^2 = 0.871,

Table 1. Antimicrobial activity of the extract of *S. pilifera*.

Microorganism	MICof extract	MICof reference[a]	Zone of Inhibition of the extract in mm (Mean ± SD)	Zone of Inhibition of the reference mm (Mean ± SD)[b]
Bacillus pumilus	128	16	12± 0.82	16.5± 1.8
Escherichia coli	128	8	9.7 ± 0.69	17.8± 0.38
Kokuria varians	256	32	12.2± 0.86	17.2 ± 0.38
Listeria monocytogenes	128	64	8.3 ± 1.02	15.4 ± 0.38
Pseudomonas aeroginosa	170.66	32	9.5 ± 0.3	19.2 ± 0.76
Salmonella typhi	128	16	13.1 ± 2.37	20.6 ± 0.67
Aspergillus flavus	256	64	6.4 ± 0.69	23.3 ± 0.33
Candida glabrata	426.66	64	6.7± 1.8	24.1 ± 0.19
Aspergillus niger	512	32	6.4 ± 0.11	23.7 ± 0.48

[a]Ampicillin, Tetracycline and Fluconazole were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively. [b]The values represent the mean of four experiments ± SD. Ampicillin, gentamicin and ketoconazole (10 lg/disc) were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively.

Table 2. Antioxidant activity and total phenolic contact of the extract of *S. pilifera.*

Plant name	DPPH IC_{50} (mg/ml)	Total phenolic content (mg catechin equivalent/g extract)
S. pilifera	2.8 ± 0.1	176.74 ± 1.93

Values represent the mean of three experiments ± SD.

$p < 0.0001$). This means that phenolic compounds provide the major contribution to the antioxidant activity of the plant extracts measured by these assays. This is in line with the observation of other authors who found similar correlations between total phenolic content and antioxidant activity of various plants (Nencini et al., 2007).

DPPH radical- scavenging activity

$DPPH^{\bullet}$ is a free radical compound and has been widely used to test the free radical scavenging ability of various samples (Sakanaka et al., 2005; Shimoji et al., 2002). It is a stable free radical with a characteristic absorption at 517 nm, was used to study the radical-scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. To evaluate the scavenging effects of DPPH of butanolic extract, DPPH inhibition was investigated. Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH•, thus neutralizing its free radical character (Naik et al., 2003).The colour changes from purple to yellow and its absorbance at wavelength 517 nm decreases (Table 2).

Phenolic compounds analysis

Phenolic compounds due to their antioxidant activities and free radical-scavenging abilities, are widely distributed in plants (Li et al., 2006), which have gained much attention and potentially have beneficial implications for human health (Govindarajan et al., 2007). Total phenol content of this extract was determined using the Folin–Ciocalteu technique (Singleton and Rossi, 1965) Briefly, a 50 µl aliquot of extract was assayed with 250 µl Folin reagent and 500 µl sodium carbonate (20%, w/v). The mixture was vortexed and diluted with water to a final volume of 5 ml. After incubation for 30 min at room temperature, the absorbance was read at 765 nm in a cuvette of 1 cm and total phenols in the ethanol extract were expressed as gallic acid equivalents (GAE), using a calibration curve of a freshly prepared gallic acid solution. Total phenol content (TPC) was determined in comparison with standard galic acid and the results expressed in terms of µmol for example galic acid/g extract (Table 2).

Antitumor activity

The extract of *stachys pilifera* has been tested against two human cancer cell lines: colon carcinoma (TH-29) and breast ductal carcinorma (T47D). The IC_{50} cytotoxicity values of the extract were compared to those found for the starting organic bases as well as for some of the anticancer agents used nowadays, that are cisplatin and oxaplatin compounds. The general method

Table 3. Antitumor activity of *Stachys Pilifera.*

Compound	Concentration(M)	IC_{50}(µg/ml) [a]	
		HT-29	T47D
S. Pilifera	0.1	45.09 ± 03.09	100.56 ± 09.81
	0.01	168.09 ± 11.90	209.81 ± 18.21
	0.001	391.09 ± 21.09	430.09 ± 13.09
Cisplatin	0.1	125.71 ± 8.09	170.59 ± 9.43
	0.01	197.06 ± 17.09	211.34 ± 17.05
	0.001	243.09 ± 12.24	277.48 ± 20.66
Oxaplatin	0.1	304.12 ± 01.09	212.31 ± 06.99
	0.01	409.98 ± 09.89	290.05 ± 13.06
	0.001	527.67 ± 18.91	359.09 ± 14.23

[a] Key to cell lines employed: colon carcinoma (HT-29) and breast ductal carcinorma (T47D).

used for testing on antitumor properties of these compounds is the standard testing method that has been previously described in greater detail (Kim et al., 2004). The compounds were first dissolved in DMSO and then filtrated. After preincubation lasting for 24 h at 37℃ in 5% CO2 atmosphere and 95% humidity the tested compounds in the concentration ranges of 0.1, 0.01, 0.001 M for extract and two control compounds. The incubation lasted for 72 h and at the end of this period IC_{50} of the dead cells and live cells were measured by trypan blue. All tests and analysis were run in triplicate. Cytotoxicity has been expressed as the concentration of extract inhibiting cell growth by 50% (IC_{50}±SD). These values showed that the compounds concentrations lethal for 50% of the tumor cells which were determined both in controls and in extract concentrations lethal for both in compounds-treated cultures (Table 3).

Conclusion

The results obtained indicate that *S. pilifera* may become important in the obtainment of a noticeable source of compounds with health protective potential, antitumor, antioxidant and antimicrobial activity.it showed mildly significant activity against Gram-positive and Gram-negative bacteria, and fungi. The results of antioxidant activity show that phenolic compounds provide the major contribution to the antioxidant activity of the plant extracts measured by these assays. This is in line with the observation of other authors who found similar correlations between total phenolic content and anti-oxidant activity of various plants. The results of antitumor activity show that this plant exhibit antitumor properties and it is important to note that they show enhanced inhibitory activity compared to control compounds. It has also been proposed that concentration plays a vital role in increasing the degree of inhabitation.

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REFERENCES

Bandoniene D, Venskutonis PR, Gruzdiene D, Murkovic M (2002). Chemical composition of essential oils of Salvia limbata from two different regions in Iran and their biological activities, Eur. J. Lip. Sci. Technol., 104: 286.

Bauer AW, Kirby WMM, Sherries JC, Turck M (1966). Antibiotic susceptibility testing by single disk method. Am. J. Clin. Pathol., 45: 493–496.

Brown D (1995).The Herb Society of America encyclopedia of herbs and their uses. London: Dorling Kindersley; p. 205.

Essawi T, Srour M (2000). Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol., 70: 343.

Ghahreman A (1995). Flora of Iran. Tehran: Research Institute of Forest an Rangelands and Tehran University Press.

Govindarajan R, Singh DP, Rawat, AKS (2007). High-performance liquid chromatographic method for the quantification of phenolics in 'Chyavanprash' a potent Ayurvedic drug. J. Pharm. Biomed. Anal., 43: 527–532.

Grice HC (1986). Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. Food Chem. Toxicol., 24: 1127.

Hayashi K, Nagamatsu T, Ito M, Hattori T, Suzuki Y (1994a). Acteoside, a component of Stachys sieboldii MIQ, may be a promising antinephritic agent (1): Effects of acteoside on crescentic-type anti-GBM nephritis in rats. Japan. J. Pharmacol., 65: 143–151.

Hayashi K, Nagamatsu T, Ito M, Hattori T, Suzuki Y (1994b). Acteoside, a component of Stachys sieboldii MIQ, may be a promising antinephritic agent (2): Effects of acteoside on leukocyte accumulation in the glomeruli of nephritic rats. Japan. J. Pharmacol., 66: 47–52.

Heath HB (1981). Source book of flavours. Westport: Avi, pp. 890.

Janssen AM, Scheffer JJC, Svendsen AB (1987). Antimicrobial activity

of essential oils a literature review aspects of the test methods. J. Planta Med., 53: 395–398.
Kim YS, Song R, Chung HC, Jun MJ, Sohn YS (2004). Coordination modes antitumor activity: synthesis and antitumor activity of novel platinum(II) complexes of N-substituted amino dicarboxylic acids. J. Inorg. Biochem., 98: 98–104.
Li BB, Smith B, Hossain MM (2006). Extraction of phenolics from citrus peels I. Solvent extraction method. Sep. Purific. Technol., 48: 182–188.
Moure A, Cruz JM, Franco D, Dominguez JM, Sineiro J, Dom´ınguez H, N´u˜nez MJ, Paraj´o JC (2001). Natural antioxidants from residual sources.J. Food Chem., 2: 145–171.
Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni PP, Biyani MK (2003). Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. J. Phytochem., 63: 97–104.
Nencini C, Cavallo F, Capasso A, Franchi GG, Giorgio G, Micheli L (2007). Evaluation of antioxidative properties of Allium species growing wild in Italy. J. Phytother. Res., 2: 874-878.
Piozzi F, Savona G, Hanson JR (1980). Kaurenoid diterpenes from *Stachys lanata*. Phytochem., 19: 1237–1238.
Rechinger KH (1982). Flora iranica, No. 150, LBIATAE. (pp. 354–396). Graz: Akademische Druck-u, Verlagsanstalt.
Ross SA, Zinchenko TV (1975). Study of triterpenoids and steroids of Stachys palustris L. Farm-Zh-Kiev (Farmatsevtichnii-Zhurnal), 30: 91–92.
Sakanaka S, Tachibana Y, Okada Y (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). J. Food Chem., 89: 569–575.
Savchenko VM, Khvorostinka VM (1978). Effects of a preparation from Stachys inflata on the course of experimental hepatitis in rats. Farm-Zh-Kiev (Farmatsevtichnii-Zhurnal), 33: 50–53.
Shimoji Y, Tamura Y, Nakamura Y, Nanda K, Nishidai S, Nishikawa Y (2002). Isolation and identification of DPPH radical scavenging compounds in kurosu (Japanese unpolished rice vinegar). J. Agric. Food Chem., 50: 6501–6503.
Skaltsa HD, Lazari DM, Chinou IB, Loukis AE (1999). Composition and antibacterial activity of the essential oils of Stachys candida and S. chrysantha from southern Greece [letter]. Planta Medica, 65: 255–256.
Vundac VB, Males Z, Plazibat M, Golja P (2005). HPTLC Determination of Flavonoids and Phenolic Acids in Some Croatian Stachys Taxa. J. Planar Chromatogr., 18: 269-273.
Wichi HP (1988). Enhanced tum from the perspective effect on forestomach and oesophageal squamous epithelium. Food Chem. Toxicol., 26: 717.
Yamahara J, Kitani T, Kobayashi H, Kawahara Y (1990). Studies on Stachys sieboldii MIQ. II. Anti-anoxia action and the active constituents. Yakugaku Zasshi, 110: 932–935.
Yamamoto R, Miyase T, Ueno A (1994). Stachyssaponins I–VIII, new oleananetype triterpene saponins from Stachys riederi Chamisso. Chemical and Pharmaceutical Bulletin, 42: 1291–1296.
Yin J, Yang G, Wang S, Chene Y (2006). Purification and determination of stachyose in Chinese artichoke (*Stachys Sieboldii Miq*.) by high-performance liquid chromatography with evaporative light scattering detection. Talanta, 70: 208-212.
Zargari A (1992). Medicinal plants. Tehran: Tehran University Press.
Zinchenko TV, Voitenko GN, Lipkan GN (1981). Anti-inflammatory, antitoxic, and hypoazotemic effect of a Stachys recta preparation, stachyrene. Farmakologiia and Toksikologiia, 44: 191–194.