ICC
Iranian Chemical Communication
Original Research Article
Payame Noor University http://icc.journals.pnu.ac.ir

# GC-MS analysis of phytocomponents and antioxidant, antimicrobial activities of aerial parts of *Stachys turcomanica*

**Majid Halimi[a,*], Malihe Nasrabadi[a], Hamid Soorgi[b], Mohabat Nadaf[c]**

[a]*Department of Chemistry, Payame Noor University , P.O. BOX 19395-3697 Tehran ,Iran*

[b]*MD. Associate Professor of Dermatology. NKUMS, Iran*

[C]*Department of Biology, Payame Noor University, P.O. BOX 19395-4697 Tehran, Iran*

**Received: 19 October 2015, Accepted: 26 January 2016, Published: 26 January 2016**

## Abstract

The aim of this study was to investigate the *stachys turcomanica* phytochemical compounds, antimicrobial and antioxidant activity of methanolic extract. The volatile constituents from the aerial parts of *Stachys turcomanica* growing wild in Iran were obtained by hydrodistillation and analyzed by GC and GC-MS. In the GC-MS analysis, Forty-six components representing 81.1% of the oil were identified. The main constituents of the oil were 1-octen-3-ol (13.4%), β -pinene (7.9%), α -pinene (5.6%), α -bisabolol(4.4%), ar-curcumene(4.0%) and β –myrcene(3.7%).The phytochemical analysis revealed the presence of alkaloids, flavonoids and terpenoid in varying concentration . The antioxidant activity of aerial parts of methanolic extract was studied *in vitro*by 2'2'-diphenylpicrylhydrazyl (DPPH) radical–scavenging activity. The methanolic extract of *stachys turcomanica* leaves exhibited amaximum DPPH scavenging activity of (81.61±0.78) %at10mg/mL followed by aerial parts of plant. Whereas for BHT (standard) was found to be (94.79±0.75) % at the same concentration . Methanol solvent was used to screen the antimicrobial activity of the selected phytopathogens by agar diffusion method. The maximum antibacterial (phytopathogene) activities were observed on *Xanthomonascampestrispv.Campestris*,*Agrobacterium sp*.and *Pseudomonas viridiflava*.

**Keywords:** *Stachys turcomanica*; phytochemical; hydrodistillation; methanolic extracts; antimicrobial; antioxidant activity.

*Corresponding author: Majid Halimi
Tel: +98 (583) 2296721, Fax: +98 (583) 2296721
E-mail: majid_halimi@pnu.ac.ir

***Iran. Chem. Commun. 4 (2016) 389-398***

**Introduction**

The genus Stachys belongs to the Lamiaceae family. This family is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide. This family has an almost cosmopolitan distribution [1-2].

Stachys species have been reported in folk medicine to treat genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers. The whole plant or leaves of these species are used in phytotherapy and said to possess sedative, antispasmodic, diuretic, antibacterial [3], anti-inflammatory [4-9], antitoxic [10-11], antioxidant [12-15] and emmenagogue activities when used as ~~a~~tea [16]. Some Stachys species are used as a tonic and for stomach ailments in Anatolia [17].

Phytochemical investigation of *Stachys* species have shown the occurrence of flavonoids, diterpenes, phenyl ethanoid glycosides and saponins [18]. This paper presents phytochemicalresults, antimicrobial and antioxidant activity of the plant collected from Darkash area of Bojnord.

**Experimental**

*Plant material:*

Aerial parts of *S.turco-manica* were collected at the flowering stage from Darkash area of Bojnord, Iran, In Jun 2010, and identified at the Research center for plant sciences, Ferdowsi university of Mashhad, Iran. A voucher specimen has been deposited in the Herbarium of research center for plant science.

**Isolation of the essential oil**

Aerial parts of *S.turcomanica* were air-dried for 5 days before isolation of essential oil. The plant material (100gr) was cut into small pieces and the essential oil obtained by hydrodistillation with a Clevenger-type apparatus until there was no significant increase in the volume of the collected oil (6h). The yield of the yellow oil was 0.05 %(w/w) based on the dry weight of the plant.

**GC-MS Analysis**

GC-MS analysis of the oil from the aerial parts of the plant was performed using a Shimadzu GC-MS-QP(2010)gas chromatograph equipped with flame ionization (FID) and a RTX-5MS(95%diphenyl/5%dimethyl polysiloxan)(15m×0.25mm.i.d., film thickness 0.25µm). The oven temperature was programmed 35-280$^{o}$C at a rate of 5$^{o}$C/mm; ionization potential 70 eV; the carrier gas was helium with a flow rate of 1ml/min the sample was injected using

the split sampling technique 1:10. The percentage composition of the oil was calculated automatically from peak areas without any correction. Retention Index (RI) compounds were determined by comparing the retention times of a series of n-alkanes with linear interpolation. Identification of each component was confirmed by comparing its retention index either with those of authentic compounds or with data in the literature [19-21].

**Antioxidant activity**

The antioxidant potential of the methanolic extract was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability. The determination was performed in triplicate.

**DPPH radical scavenging activity**

The scavenging effect on the DPPH radical was deter-mined according to the methods reported earlier [8].50µl of various amounts of methanolic extracts (10, 5, 2.5, 1.125, 0.625, 0.312, 0.156 and 0.078 mg/mL) were mixed with 5 mL of 0.004% methanolic solution of DPPH. Each mixture was incubated for 30 min in the dark and the absorbance of the samples was determined at 517 nm using Micro plate reader. The DPPH solution was freshly prepared and kept in the dark at 4°C in between the measurements. Methanol and BHT were used as control and as a standard of the assay, respectively.

A lower absorbance indicated a higher radical scavenging power and was calculated according to the equation: DPPH scavenging activity (%I)= [1–($A_t$/$A_o$)] x100,where $A_t$ is the absorbance of the sample at 517nm and $A_o$ is the absorbance of the control at 517 nm.

**Antibacterial activity assay**

*Test microorganisms*

Phytopathogenic bacteria were collected from the culture collection of Department of Applied Botany and Biotechnology, University of Sari, Iran. All the test bacterial species were maintained on nutrient agar media.

Antibacterial activity of methanolic extract was determined by disc diffusion method on nutrient agar medium. Sterile Whatmann filter discs (6 mm diameter) were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing $10^6$ CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50µl methanolic extract was placed in the discs made in inoculated plates. The treatments also included 50 µl of solvents served as control and chloramphenicol as a standard

control. The plates were incubated for 24 h at 37 ºC and zone of inhibition if any around the wells were measured in mm (millimeter). Each treatment consists of three replicates and repeated at least twice. Minimum inhibitory concentration (MIC), which was determined as the lowest concentration of S.turcomanica plant extract inhibiting the growth of the organism, was determined based on the readings [22].

**Antimicrobial testing**

●Prepare MHA medium, cool the medium to 50 ˚C, Add antibiotic to final concentration range between 0.125 and 128 mg $l^{-1}$

●Pour a control agar plate without any antibiotic

●Dry the surface of the agar plates in the laminar air flow hood for 30 min.

●Mark the bottom of the agar plates to define an orientation.

●Mix the bacterial suspension by vortexing and dilute it 1:10 into a sterile plate by pipetting 10 µl into a well containing 90 µl of sterile broth or saline.

●Deliver 1 µl of the diluted suspension to the each agar medium surface.

●Let the inoculum spots dry at room temperature before inverting the plates.

●Incubate agar plates at 37 ˚C for 16–20 h [22].

**MIC determination**

●Check the antibiotic-free growth control plate.

●Determine the lowest concentration of the antimicrobial substance that inhibits visible growth of the tested isolate [22], (Table 4).

**Results and discussion**

*Phytochemical screening*

The present study which was carried out on the *Stachys turcomanica* revealed the presence of medicinal active constituents. To identify the phytochemical derivatives in the methanolic extract, standard phytochemical screening was performed [23, 24]. Alkaloids test was performed by Dragendorff's and Meyer's tests, flavonoidsby Pew's and Shinoda's tests, saponin by frothing test, test for terpenoids by Salkowski test and tannins by FeCl3 and lead acetate [25-27].

The phytochemical active compounds of *Stachys turcomanica*were qualitatively analyzed and the results are presented in Table 1. In this screening process Alkaloid, Flavonoids and Terpenoid gave positive results.

**Table 1.** Phytochemical analysis aerial parts of extract of *Stachys turcomanica*

| Phytochemical components | *Stachys turcomanica* |
|---|---|
| Alkaloid | + |
| Flavonoid | + |
| Saponin | _ |
| Tannin | _ |
| Terpenoid | + |

**GC-MS analysis**

From the aerial parts of *Stachys turcomanica* at flowering stage, yellowish oil was obtained at a yield of 0.05 %( w/w).Forty-six components were identified, accounting for 81.1% of the total oil. The oil of *Stachys turcomanica* was characterized by1-octen-3-ol (13.4%),β-pinene (7.9%), α-pinene (5.6%), α-bisabolol(4.4%), ar-curcumene(4.0%),β–myrcene(3.7%). The percentage composition of the various oil components are listed in Table 2.

The various compounds were identified by comparison of their Kováts retention indexes, determined utilizing a non-logarithmic scale on non-polar (Rtx-5MS) columns, and by comparison of the mass spectra of each GC component with those of standards and with reported data. Table 2 shows the distribution of *Stachys turcomanica* compounds.

**Table 2.** Percentage composition of the essential oil isolated from aerial parts of *Stachys turcomanica*by GC-MS

| No. | Compound | Retention Index (RI[a]) | Percentage |
|---|---|---|---|
| **1** | 3-hexen-1-ol | 841 | 0.4 |
| **2** | P-xylene | 883 | 2.7 |
| **3** | α –thujene | 923 | 0.4 |
| **4** | α –pinene | 939 | 5.6 |
| **5** | 2-propenyl-benze | 947 | 0.4 |
| **6** | β –pinene | 964 | 7.9 |
| **7** | 1-octen-3-ol | 919 | 13.4 |
| **8** | β –myrcene | 953 | 3.7 |
| **9** | δ -3-carene | 1008 | 1.1 |

| | | | |
|---|---|---|---|
| **10** | p-cymene | 1069 | 2.1 |
| **11** | dl-limonene | 1081 | 2.7 |
| **12** | 1,8-cineole | 1093 | 1.0 |
| **13** | γ -terpinene | 938 | 0.4 |
| **14** | Octylformate | 967 | 1.5 |
| **15** | L-fenchone | 976 | 0.4 |
| **16** | Linalool | 999 | 2.9 |
| **17** | Nonanal | 1107 | 0.5 |
| **18** | Terpinene-4-ol | 1172 | 0.6 |
| **19** | α -terpineol | 1054 | 0.4 |
| **20** | Methyl salicylate | 1060 | 0.7 |
| **21** | Fenchyl acetate | 1216 | 0.8 |
| **22** | Carvacrol | 1298 | 1.1 |
| **23** | Cis-isoeugenol | 1357 | 0.3 |
| **24** | α -copaene | 1369 | 1.0 |
| **25** | β -bourbonene | 1377 | 0.7 |
| **26** | β -elemene | 1387 | 1.0 |
| **27** | β -caryophyllene | 1411 | 1.0 |
| **28** | aromadendrene | 1454 | 0.2 |
| **29** | Germacrene-D | 1475 | 1.8 |
| **30** | ar-curcumene | 1480 | 4.0 |
| **31** | Valencene | 1487 | 2.1 |
| **32** | β -bisabolene | 1506 | 0.3 |
| **33** | Selina-3,7(11)-di | 1510 | 1.5 |
| **34** | δ -cadinene | 1520 | 1.3 |
| **35** | spathulenol | 1571 | 0.9 |
| **36** | Caryophyllene ox | 1576 | 0.7 |
| **37** | veridiflorol | 1585 | 1.4 |
| **38** | Globulol | 1596 | 0.6 |

| | | | |
|---|---|---|---|
| **39** | γ –cadinene | 1638 | 0.7 |
| **40** | t-muurolol | 1650 | 2.3 |
| **41** | α –farnesene | 1685 | 0.5 |
| **42** | α –bisabolol | 1683 | 4.4 |
| **43** | 6,10,14-trimethyl 2-pentadecanone | 1847 | 0.q5 |
| **44** | dibutyl phthalate | 1870 | 2.1 |
| **45** | Phytol | 2115 | 0.9 |
| **46** | diisooctyl-phthalate | 2552 | 0.2 |
| | | Total | 81.1 |

a: Retention Indices on RTX-5MS(based on homologous series of n-alkanes;$C_8$-$C_{24}$

**DPPH assay**

Whereas for BHT (standard) was found to be (94.79±0.75) % at the same concentration, the methanolic extract of *stachys turcomanica* leaves exhibited amaximum DPPH scavenging activity of (81.61±0.78) %at10mg/mL followed by aerial parts of plant.

**Antibacterial activity**

The results of antibacterial activity of Methanolic extract of *Stachys turcomanica* at various concentrations are shown in Table 3. Methanolicextract was subjected to antibacterial activity and showed significant activity on this bacteria.The maximum antibacterial activities were observed on Xanthomonascampestrispv.campestris, Agrobacteriumsp.and Pseudomonas viridiflava. bacteria for the most common plant diseases and antioxidant activity.

**Table 3.** Minimum inhibitory concentration (MIC) of methanolic extract of *stachys turcomanica* (%v/v) against microorganisms

| **Concentration** | **X.a** | **X.c** | **R.r** | **R.t** | **A.sp** | **P.s** | **P.v** |
|---|---|---|---|---|---|---|---|
| 1mg/l | - | - | - | - | - | - | - |
| 5mg/l | - | - | - | - | - | - | - |
| 10mg/l | - | 10mm | - | - | 9mm | - | 8mm |

***X.a****: Xanthomonasarboricola,* ***X.c****: Xanthomonascampestrispv,* ***R.r****: Rathayibacterrathayi,* ***R.t****: Rathayibactertritici,* ***A.sp****: Agrobacterium* sp, ***P.s***: *Pseudomonas syringaepv*, ***P.v***: *Pseudomonasviridiflava*

**Table 4.** Lowest concentration of the antimicrobial substance that inhibits visible growth of the tested isolate

| ***Bacteria*** | **Disease** | ***1mg/l*** | ***10mg/l*** | ***100mg/l*** | ***500mg/l*** |
|---|---|---|---|---|---|
| *Pseudomonas viridiflava ( G $^-$)* | Bacterial leaf spot | + | - | + | + |
| *Pseudomonas syringaepv.( G $^-$)* | bacterial canker | + | - | + | + |
| *Rathayibacterrathayi (G$^+$)* | cause galls or gummosis | - | - | + | + |
| *Xanthomonascampestrispv.( G $^-$)* | black rot | + | - | + | + |
| *Xanthomonasarboricola (G $^-$ )* | Bacterial leaf blight | + | - | + | + |
| *Agrobacterium* sp.(*G $^-$* ) | Crown Gall | + | - | + | + |
| *Rathayibactertritici (G $^+$ )* | yellow ear rot(Bacterial Spike Blight) | + | - | + | + |

**Conclusion**

The GC-MS analysis of *stachys turcomanica* essential oil revealed the presence of 46 compounds. The high content of 1-octen-3-ol (13.4%), β -pinene (7.9%), α-pinene (5.6%),α-bisabolol(4.4%),ar-curcumene(4.0%), β–myrcene(3.7%) are presented in the essential oil from the *stachys turcomanica*. The phytochemical studies of the arial parts of the *stachys turcomanica* as well as the biological activities of the resulting essential oils are currently under investigation in our laboratories. Methanol extract of *stachys turcomanica*possess a broad spectrum of activity against a panel of phytopathogenic bacteria for the most common plant diseases and antioxidant activity.

The highest DPPH scavenging activity has showed (81.61±0.78) % at a concentration of 10 mg/ml. While BHT (standard) shown to be (94.79±0.75)% at the same concentrations. Maximum antibacterial activity was shown on Xanthomonascampestrispv. campestris Agrobacterium sp. and Pseudomonas viridiflava.

The rich diversity of the plants has encouraged the screening and scientific validation of the plant extracts for antimicrobial and antioxidant properties which leads to the discovery of many new antimicrobial and antioxidant drugs of plant origin. In the present investigation, the antibacterial activity and antioxidant of *stachys turcomanica* has been demonstrated for the first time and which has resulted in identifying a new candidate plant for controlling plant diseases. Further research is necessary to isolate and determine the identity of the active compound.

**Acknowledgments**

The authors are grateful to Mr. Joharchi and Mr. Memariani for identification of the plant material, Payame Noor University of Iran for the financial support and Sharif University for GC-MS.

**References**

[1] Hedge, I.C., A global survey of the biogeography of the Labiatae. In Harley R.M. Reynolds T., Advances in Labiatae Science. Royal Botanical Gardens, Kew, London, **1992**.

[2] Hedge, I.C., Labiatae of South-west Asia: diversity, distribution and endemism, Procedings of the Royal Society of Edinburg., **1986**, 89, 23-35.

[3] H.D. Skaltsa, DM. Lazari, IB.Chinou, AE. Loukis, *Planta Med*., **1999**, 65, 255–256.

[4]M. Khanavi, M. Sharifzadeh, A. Hadjiakhoondi, A. Shafiee, *J. Ethnopharmacol*., **2005**, 97, 463-8.

[5] M. Khanavi, M. Sharifzadeh, A.Hadjiakhoondi, A. Shafiee, *Iranian J. Pharm. Res*., **2004**,2, 55-56.

[6] J. Kukic, S. Dobric, S. Petrovic, *Pharm. Biol*., **2007**, 45, 560-3.

[7] N. Maleki, A. Garjani, H. Nazemiyah, N. Nilouroushan, A.T. Eftekhar Sadat, Z. Allame, N. .Hasannia, *J. Ethnopharmacol*., **2001**, 75, 213-8.

[8] M. Sharifzadeh, K. Sharifzadeh, M.Khanavi, A. Hadjiakhoondi, A.Shafiee, *Int. J. Pharmacol*., **2005**, 1, 132-7.

[9] H.D. Skaltsa, P. Bermejo, D.M. Lazari, A.M. Silvan, A.L. Skaltsounis, A. Sanz, M.J. Abad, *Biol. Pharm. Bull*., **2000**, 23, 47-53.

[10] T. V. Zinchenko, G. N. Voitenko, and G. N.Lipkan, *Farmakol Toksikol.*, **1981**,44,191-4 .

[11] N. Maleki, A. Garjani, H. Nazemiyeh, N. Nilfouroushan, A. T. Eftekhar Sadat, Z. Allameh, N. Hasannia , *J. Ethnopharmacol*., **2001**, 275, 213-218.

[12] J. Kukić, S. Petrović, M. Niketić, *Biol. Pharm. Bull.*, **2006**, 29,725-729.

[13] M. Couladis, O. Tzakou, E. Verykokidou, C. Harvala, *Phytother. Res*., **2003**, 17, 194-195.

[14] D. Mantle, F. Eddeb, A. T. Pickering, *J. Ethnopharmacol*., **2000**, 72, 47-51.

[15] F. Conforti, F. Menichini, C. Formisano, D. Rigano, F. Senatore, N. A. Arnold, F. Piozzi, *Food Chem.*, **2009**, 116 ,898-905.

[16] L.G. Miller, W.J. Murray, Herbal Medicinals. A Clinician's Guide. Pharmaceutical Products Press: Binghampton, NY, **1998**, 223.

[17] T. Baytop, Therapy with Medicinal Plants in TurkeyPast and Present (in Turkish), 2nd ed. Nobel Tip Basimevi: Istanbul, **1999**,193.

[18]M. Khanavi, M. Sharifzadeh, A. Hadjiakhoondi, A. Shafiee, *J. Ethnopharmacol.*, **2005**, 97, 463–468.

[19] W. Jennings and J. Shibamato, Qualitative analysis of flavour and fragrance volatile by capillary gas chromatography. Academic press New York, **1980**.

[20] R.P. Adams, Identification of essential oil components by Gas chromography / Mass spectrometry. Allured, Carol Stream, IL., **2001**.

[21] N.W. Davies, *J. Chromatogr*., **1990**, 503, 1-24.

[22] W. Irith Kai Hil, E.W. Robert Hancock, Nature Protocol, **2008**, 3, 163-175.

[23] J.B. Harborne: Phytochemical methods: a guide to modern techniques of plant analysis. London, Chapman and Hall, Ltd, **1973**, 2, 49-188.

[24] G.E. Trease, W.C. Evans: Orders and familys of plant in pharmacognosy. Oxford University Press, **1983**, 12, 343-383.

[25] Sofowora A: Recent trends in research into African medicinal plants. *J. Ethnopharmacol*, **1993**, 38(2-3):209-214.

[26] A. Sofowora, *J. Altern Complement Med*., **1996**, *2*, 365-372.

[27] D. Krishnaiah, T. Devi, A. Bono and R. Sarbatly, *J. Med Plants Res*., **2009**, 3, 67-72.