Journal of Medicinal Plants Research Vol. 6(16), pp. 3105-3109, 30 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1270
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Chemical composition of essential oil, antibacterial activity and brine shrimp lethality of ethanol extracts from *Sedum pallidum*

Abbas Ali Dahpour, Parvaneh Rahdari* and Zahra Sobati

Department of biology, Tonekabon beanch, Islamic Azad University, Tonekabon, Iran.

Accepted 15 February, 2012

The essential oils of *Sedum pallidum* is obtained by hydro distillation and analyzed by (GC/MS) for determining their chemical composition and identification of their chemo types. The major component was Hexadecanoic Acid (33.5%), other predominant components were Phenol (9.96%), Eicosane (9.19%), Cyclotrisiloxane, hexamethyl (4.78%), Oxime-methoxy-phenyl (4.34%), Neophytadiene (4.32%), Phytol (3.86%), 3-alpha H-2- Oxofurano (2.78%). The extract of *Sedum pallidum* (400 mg/disc) showed moderate activity against three bacteria. The zone of inhibition against *Proteus mirabilis*, *Enterobacter Cloacae* and *Staphylococcus aureus* was 10.11 and 18 mm diameter, respectively. But, the extract of *S. pallidum was not* activity against on *Bacillus subtillis* and *Klebsiella pneumonia*. The positive control, Ampicilin, Gentamicine and Stereptomaicin had shown zone of inhibition resistant of all bacterial. The brine shrimp lethality activities of ethanolic extracts of *S. pallidum* were evaluated in this study. The results of cytotoxic activity of these extracts were more active against brine shrimp lethality of *Artemia salina*.

Key words: *Sedum pallidum*, essential oil, antibacterial activity, brine shrimp lethality.

INTRODUCTION

In recent years, an upsurge of interest in the use of natural substances as phytomedicines has resulted in an ore thorough investigation of plant resources. Plants have played a key role in the worldwide maintenance of health. Infectious diseases remain the leading cause of death worldwide and infections due to antibiotic resistant microorganisms have become more widespread in recent years (WHO 2001). Aromatic plants and their essential oils, used since antiquity in folk medicine and for the preservation of food, are known sources of natural secondary metabolites having biological activity such as antimicrobial and antioxidant activity among many others (Deans and Svoboda, 1990). Among many recent advances in cancer chemotherapy, photochemical play an important role in cancer chemotherapeutic drugs. A search for new anti-cancer drugs has taken many different approaches. The brine shrimp lethality bioassay is efficient, rapid and inexpensive testes that require only a relatively small amount of samples. The technique is easily mastered, costs little, and utilizes small amount of test material (Meyer et al., 1982) has been successively employed for *in vivo* lethality bioassay-guide fractionation of active cytotoxic and antitumor agents such as trilobacin from bark of *Asimina triloba* (Zhao et al., 1992), *cis*-annonacin from *Annona muricata* (Reiser et al., 1996) and ent-kaur-16-en-19-oic acid from Elaeoselinum foetidum (Mongelli et al., 2002). *Sedum pallidum* is a species from the family of *Crassulaceae*. It is distributed extensively in the Northwest and North of Iran (flora Iranica).

Among all the *Sedum* species that have been investigated, *S. pallidum* has not received great attention. Considering the ethno pharmacological profile of this genus, the aim of this work was to assess the antinociceptive and anti-inflammatory activities and the chemical composition of that our first species. Here we report result of chemical and pharmacological ingestions undertaken with a fresh juice from leaves of *S. pallidum.*

*Corresponding author. E-mail: rahdari_parvaneh@yahoo.com.

Previously, composition essential oil, antimicrobial and brine shrimp lethality activity has not been investigated. The present study was work carried out on the preliminary phytochemicals identification, antibacterial activity and cytotoxicity properties of *S. pallidum*.

MATERIALS AND METHODS

Plant materials

The flowers of *S. pallidum* were collected from Chamestane *Nour* Iran (North of Iran), in the summer of 2010. The samples were identified by Dr. Bahman Eslami (Assistant. Prof. of plant systems, Islamic Azad University of Qaemshahr, Iran). Voucher specimens are deposited with the Faculty of Biology Herbarium (No 692 to 696).

Isolation of the essential oil

The flowers of the plant collected were submitted for 3 h to water-distillation using a British-type Clevenger apparatus. The obtained essential oil was dried over anhydrous Sodium sulfate and after filtration, stored at 4°C until tested and analyzed.

Gas chromatography–mass

Spectrometry (GC – MS)

GC – MS was carried out using a Hewlett-Packard 5975B series instrument and an Agilent 19091J-433 HP-5 capillary column (30 m., 250 m i.d., film thickness 0.25 m) which was set at 50°C for 10 min, then increased 4°C/min to 300°C; using Helium as a carrier gas at a flow rate of 1 ml/min. The split ratio was 1:10; ionization energy was 70 eV; scan time was 1 s; acquisition mass range was m/z 40 to 400. The compounds were identified according to their retention indexes and by comparison of their mass spectra with those of a computer library or with authentic compounds. α-pinene, Decane, Benzene and Limonene were identified by co-injection.

Preparation of ethanolic extract

The flowers were dried at room temperature before extraction. A known amount of flowers were extracted at room temperature following the percolation method using Ethanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained (10.8%), which was then freeze-dried for complete solvent removal.

Assay for antibacterial activity of plant extract

Antibacterial activity of plant extract was determined by disc diffusion method as described by (Bauer et al., 1966). Three Gram (-) bacteria (*Proteus mirabilis PTCC (1076); Enterobacter cloacae PTCC (1003); Klebsiella pneumonia PTCC (1290)),* and two Gram (+) bacteria (*Staphylococcus aureus PTCC (1112)*) and *Bacillus subtilis PTCC (1023)*) were used for the present study. All the tested bacteria were collected from Pastor Institute of Iran. Dried filter paper discs (4 mm in diameter) impregnated in known amount of test substances (400 µg/discs) were placed on Mueller-Hinton agar medium uniformly seeded with the test organisms. Valinomycine, Gentamicine and Cholrampnicol discs (30 µg/disc) soaked in respective solvent were used as positive control. These plates were then kept at low temperature (4°C) for 2 to 4 h to allow maximum diffusion of compound. The diffusion occurred according to the physical law that controls the diffusion of molecules through agar gel the plates were then incubated at 37˚C for 24 h to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disc called “Zone of Inhibition”. The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Brine shrimp lethality assay

In vitro lethality assay of *Artemia salina* was used to detect cell toxicity (Meyer et al., 1982). Brine shrimp eggs were placed in seawater (3.8% w/v sea salt in distilled water) and incubated at 24 to 28°C in front of lamp. Eggs were hatched with in 48 h providing large number of larvae (nauplii).

A convenient number of nauplii were placed in vials containing 5 ml of seawater and increasing concentrations of *S. pallidum* extract. Control was made with the same volume of 96% ethanol in seawater without addition of *S. pallidum* extract. A live nauplii were counted after 16 h and the lethal concentration (LC_{50}) was calculated.

Statistical analysis

Lethality assays were evaluated by Finney computer statistical program to determine the LC_{50} values and 95% confidence intervals. All other data were expressed as percentage.

RESULTS

Chemical composition of the essential oil

The results obtained by the GC-MS analysis of the essential oil of the. *S. pallidum* aerial parts are presented in Table 1. Forty compounds were identified, representing 98.8% of the total oil. The oil yield of the plant was determined as 0.94% v/w. As determined from the GC-MS analysis.

The major compounds were Hexadecanoic Acid (33.5%), Phenol (9.96%), Eicosane (9.19%), Cyclotrisiloxane, hexamethyl (4.78%), Oxime-,methoxy-phenyl (4.34%), Neophytadiene (4.32%), Phytol (3.86%) and 3. alpha H-2- Oxofurano (2.78%).

Assay for antibacterial activity

Crude extract of *S. pallidum* (400 mg/disc) showed moderate activity against three bacteria. The zone of inhibition against *P. mirabilis*, *E. cloacae* and *S. aureus* was 10.11 and 18 mm diameter, respectively. But, the extract of *S. pallidum* was not activity against on

Table 1. Chemical composition of the essential oil of *Sedum pallidum.*

S/N	K.I	Components	Composition (%)
1	986	Benzonitrile,m-phenethyl	1.15
2	1013	Hydroxyl-2-phenylacetonitrile	0.43
3	1069	Oxime-,methoxy-phenyl	4.34
4	1104	n-methyl-1,3-dithioisoindoline	0.83
5	1146	Cyclotetrasiloxane,octamethyl	1.44
6	1258	7methyl-2 phenylindole	0.42
7	1299	Pentadecane,2,6,10,14-tetramethyl	0.53
8	1303	2-cyclohexen-1-one,3,5,5-trimethy	1.89
9	1346	Cyclopentasiloxaane,decamethyl	0.55
10	1398	Gamma-terpinene-1/4 cyclohexadiene	0.25
11	2496	Cyclotrisiloxane,hexamethyl	4.78
12	1400	Tetrasiloxane	0.05
13	1576	cyclohexasiloxane	0.52
14	2849	Eicosane	9.19
15	1810	n-ethyl-1,3-dithioisoindoline	0.14
16	1814	Arsenous acid	0.35
17	1840	Phenol	9.96
18	1952	hexadecane	0.55
19	2051	6-Aza-5,7,12,14-tetrathiapentacene	0.40
20	2103	Pentacosane	0.87
21	2115	n-ethy-1/3-dithioisoindoline	0.37
22	3279	Tetrasiloxane,decamethyl	1.58
23	2217	Gibberellin A3	0.88
24	2221	3-Quinolinecarboxylic	1.29
25	3015	Heptamethyl trisiloxa	0.48
26	2359	Neophytadiene	4.32
27	2307	2-pentadecanone	2.69
28	2248	Dibuthyl phthalate	1.26
29	2483	Octadecane	2.36
30	2425	Silane	0.99
31	2459	4,methyl-2phenyljndole	0.27
32	2539	Hexadecanoic Acid	33.5
33	2694	N-ethyl	0.35
34	2745	Phytol	3.86
35	3083	Nonadecane	0.88
36	3097	1/3-Dioxan	1.14
37	3103	Odecen	0.18
38	3303	2/4 Dimethyl	1.68
39	3334	3.alpha.H-2-Oxofurano	2.78
40	3359	N-ethyl-1/3 dithioisoindoline	0.32

B. subtillis and *K. pneumonia* on the other hand, Standard antibiotic Ampicilin, Gentamicine and Stereptomaicin showed significant antibacterial activity against all tested Gram (+) and (-) bacteria (Table 2).

RESULTS OF BRINE SHRIMP LETHALITY ASSAY

The cytotoxic activity of of extract of *S. pallidum* was investigated *in vitro* tested against the brine shrimp (*A. salina*) the results are given in Table 3. All the crude extracts of *S. pallidum* species resulting in LC_{50} values of less than 0.4 µg/ml were considered for active against brine shrimp.

DISCUSSION

Forty compounds were identified in the present study,

Table 2. Antibacterial activity of *S. pallidum* ethanol extract expressed as minimum inhibitory concentrations (MICS) in- G/ML.

Microorganisms	*S. pallidum* ethanol extract (400 µ/disc) (mm diameter)	Gentamicine (30 µg/disc) (mm dimater)	Ampicilin (30 µg/disc) (mm diameter)	Stereptomaicin (30 µg/disc) (mm dimater)
P. mirabilis PTCC(1076)	10	21	19	19
E. cloacae PTCC(1003)	11	24	9	21
K. pneumonia PTCC(1290)	0	23	0	15
S. aureus PTCC (1112)	18	21	39	11
B. subtillis PTCC(1023)	0	31	22	29

Table 3. Brine shrimp bioassay results of extract of *S. pallidum.*

Extract concentration (µg/mg)	0.1	0.2	0.4	0.6	0.8	1
Lethality percentage (%)	0	0	50	80	100	100

LC_{50}=0.4 µg/mg LC_{50} ´s were estimated using logit transformation. Therefore confidence Intervals are not provided. An average of three replicates.

representing 98.8% of the total oil. The extraction of the plant low antimicrobial activity against gram (+) and (-) bacterial. The antimicrobial activity revealed that this leaves had similar to those of *S. palliudum* var. Bithynicum and *S. spurium* essential oil analysed by (Yaylin et al., 2010) which the antibacterial activity was not observed against *Bacillus spp*. Only 25 compounds have been identified in recently published paper (Khjeh et al., 2005). E-1- propenyl sec-butyl disulfide was the major component of the plants in that report (40.0%) but this component was only a minor one in our study (0.7%). Phenol, 2-methyl-5- (1- methyl ethyl) with 18.2% and α- Bisabolol with 10.4% were the major compounds. Maybe local climate and/or the increase in temperature to 300°C (instead of 250°C in the Khajeh et al., (2005) report) have played a major role in the number of identified components. The antimicrobial activities of various plants have been reported by many researchers (Cowan, 1999; Dewanjee et al., 2008). As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids and triterpenoid are producing a better opportunity for testing wide range of microorganism. In the present study a variety of positive and negative gram strains were selected for screening antimicrobial effects of ethanolic extract of *S. pallidum*. The result of this study showed that the ethanolic extract of exhibited varied range of antimicrobial activity against the tested organism including positive and negative gram bacteria, which is comparable to standard antibiotic effect. The *S. pallidum* extracts exhibited the greatest antimicrobial activities (as determined by the diameters of the inhibition zones towards the most susceptible bacteria like *P. mirabilis*, *E. cloacae*, *K. pneumonia* and *S. aureus*. In some cases ethaolic extract showed stronger effect on Gram (+) and (-) bacteria. This bioassay has a good correlation with cytotoxic activity in several plant extracts of *Ocimum sanctum*, *Lagerstroemia reginae*, *Cissampelos pareira*, *Acacia conccina*, *Punica granatum*, *Aconitum species*, *Rosa damascene*, *Cinchona species*, *Bacopa monnieri*, *Symplocos racemosa* were showed significant lethality to brine shrimp (Krishnaraju et al., 2006). In less active against brine shrimp lethality effect of ethanolic extract of *Annona crassiflora* leaves are earlier reported (Pimenta et al., 2003). In the present reports of ethanol extract of bark and leaf of all the *S. pallidum* showed good brine shrimp lethality. Further studies should be going on fractionation and identification of bioactive constituent to human cell line culture of cytotoxic effect.

Conclusion

In summary, pharmacological evaluation of *S. pallidum* extract reveals some interesting activities like cytotoxicity and antibacterial activities of this plant. Since, crude ethanol extract of *S. pallidum* showed cytotoxicity and antibacterial effect, we assume that different active; secondary metabolites are present in its extracts and perhaps some of these compound may function in a synergistic manner. Further studies should be going on fractionation and identification of bioactive constituent to human cell line culture of cytotoxic effect. This report may serve as a footstep regarding the biological and pharmacological activities of *S. pallidum*.

REFERENCES

Bauer AW, Kirby WMM, Sherris JC, Tuck M (1966). Antibiotic susceptibility testing by single disc method. Am. J. Clin. Pathol., 45: 493-496.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microb., 22: 564-582.
Deans SG, Svoboda KP (1990). Biotechnology and bioactivity of culinary and medicinal plants. AgBiotech. News Inf., 2: 211-216.
Dewanjee S, Maiti A, Das S, Subhash RS, Mandal C (2008). *In vitro* Antioxidant activity of *Diospyros peregrinai* fruits. Int. J. Pharmacol. Biol. Sci., 2(3): 135-144.
Khajeh M, Yamini Y, Bahramifar N, Sefidkon F, Pirmoradei MR (2005). Comparison of essential oils compositions of *Ferula assa-foetida* obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Food Chem., 91: 639-644.
Krishnaraju AV, Tayi RVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV (2006). Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). Int. J. Appl. Sci. Eng., 2: 115-125.
Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982). A convenient general bioassay for active plant constituents Planta Med., 45: 31-34.
Mongelli E, Pomilio AB, Bustamante SJ, Guerra MF, Martٍnez MG (2002). *ent*-Kaur-16-en-19- oic acid, a KB cells cytotoxic diterpenoid from *Elaeoselinum foetidum*. Phytother. Res., 16(I4): 387-388.
Reiser J, Harmison G, KluepfelStahl S, Brady RO, Karlsson S, Schubert M (1996). Transduction of Non-dividing Cells Using Pseudotyped Defective High-Titer HIV Type 1 Particles. Proc. Natl. Acad. Sci. United States Am., 93(26): 15266-15271.
Pimenta SLP, Pinto GPB, Takahashi JA, Silva LGF, Boaventura MAD (2003). Biological screening of Annonceous Brazilian Medicinal plants using *Artemia salina* (Brine Shrimp Test). Phytomedicine, 10: 209-212.
Yaylin N, Yaser A, Yilmaz IN, Yayli N, Cansut B, Coskuncelebi KK (2010). Chemical constituents and antimicrobial activities of the essential oils from *Sedum pallidum* var. *bithynicum* and *S. spurium* grown in Turkey. Pharm. Biol., 48(2): 191-194.
Zhao G, Hui Y, Rupprecht JK, McLaughlin JL, Wood KV (1992). Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. J. Natl. Prod., 55: 347-356.
Who H (2001). Global Strategy for Containment of Antimicrobial Resistance. Availabe on Internet at: www.who.int/emcdocuments.