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Vol. 8(40), pp. 1208-1214, 25 October, 2014
DOI: 10.5897/JMPR2014.5557
Article Number: 6B5985E48484
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Essential oil, fatty acids and anti bacterial activity of *Sesbania punicea* from north of Iran

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Received 10 September, 2014; Accepted 17 October, 2014

Essential oil composition of the leaves and fatty acids from the seeds of *Sesbania punicea* (rattlebox) were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Forty components (91.35%) of the essential oil were identified with the major compounds: 1,8-cineole (47.58%) and α-pinene (7.30%). The main compounds from thirty seven identified components comprising 89.28% of the seeds (hexane extract) were: linoleic acid (ω-6) (6.08%) and oleic acid (2.65%). The more abundant compounds in hexane extract of the seeds were hydrocarbons (77.32%). Antibacterial activity of ethyl acetate extract from the leaves was also evaluated by disc diffusion method against 8 gr (+/-) bacteria from which, *Salmonella parathyphi* B was the most sensitive one, even more than chloramphenicol as a standard antibiotic.

Key words: 1,8-Cineole, linoleic acid, *Salmonella parathyphi* B, *Sesbania punicea.*

INTRODUCTION

Sesbania punicea (Fabiaceae) is an ornamental shrub which has a high demand for water and thrives in high moisture areas. It has been widely distributed from its native range in South America (Hoffmann and Moran, 1991). This species has been reported as an invasive species in many of the southern United States. The plant is actively replacing nature species of riparian areas, which is taking food resources away from the local wild life and contributes to riverbank erosion and flooding in areas where it persists. *S. punicea* has been declared a noxious weed and/or seed. Any animal or human that ingests this plant or seed can become very sick and may experience symptoms such as vomiting, diarrhea, respiratory failure or fatalities (Russell, 2012). The compounds contained in this plant that makes it so toxic are saponic glycosides (Graaf, 1986).

There is a few phytochemical and pharmacological study on *Sesbania* species in the literature. Although *S. punicea* has no known industrial or medicinal use, some other species of the genus have been reported for their

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biological activities. For instance, *Stephanomeria virgata* which is a close relative to *S. punicea* has been shown to reduce the response to painful stimulation as well as inflammatory edema in mice (Russell, 2012). One of the most useful species of the genus is *Sesbania grandiflora*, which has been the subject of many projects. Sterols, saponins and tannins have been isolated from different parts of the plant (Fojas et al., 1982). These compounds are known for their biological activities such as: antibacterial and antifungal (Goun et al., 2003); antioxidant and anti urolithiatic (Doddola et al., 2008); hepatoprotective properties (Pari and Uma, 2003) and anti-tuberculosis activity (Noviani et al., 2012). Previous studies on *Sesbania* species also revealed the presence of components which possess antitumor activities. Sesbanimide, a novel anti-tumor component was isolated from the methanol extract of *Sesbania drummondii* (Hui et al., 1986) and *Sesbania vesicaria* (Kim et al., 1992). Sesbanine, a cytotoxic alkaloid with highly unusual spirocyclic structure was also isolated from the ethanol extract of *S. drummandii* seeds (Powell et al., 1979). Three new triterpenoid saponins were isolated from the seed extract of *S. vesicaria* which showed cytotoxic effect (Yuan et al., 2013). Ethanolic seed extracts of *S .vesicaria, S. punicea* and *S. drummondii* revealed significant anti tumor activities against lymphocytic leukemia P–388 (PS) in mice (Powell et al., 1976).

S. punicea (rattlebox) is cultivated in North of Iran as an ornamental plant because of beautiful reddish-orange flowers and has not been previously mentioned for chemicals and biological activities. In this project, we identified volatile compounds of leaves and fatty acids from the seeds of *S. punicea*. We also tested antibacterial activity of ethyl acetate extract of the leaves. To the best of our knowledge, this is the first report on essential oil composition and antibacterial activity of *S. punicea* leaves.

MATERIALS AND METHODS

Plant

The leaves and seeds of *S. punicea* were collected from the seaside area in Roodsar (North of Iran) in July, 2012 and were dried in shade. Identification of the plant was done by using the identification key in the Manual of Cultivated Trees and Shrubs (Bailey, 1975) and confirmed by Research Institute of Forests and Rangelands, Tehran, Iran (TARI).

Essential oil isolation

Air dried and powdered leaves (100 g) were subjected to a Clevenger-type apparatus for hydrodistillation. Then the distillate was isolated and dried over anhydrous sodium sulfate and the oil was stored at 4°C until analysis by GC and GC–MS.

Preparation of ethyl acetate extract

Ethyl acetate extract was prepared by a classic maceration method. For this purpose, air dried and powdered leaves (5 g) were soaked in ethyl acetate (30 ml) for one week at room temperature. Vigorous stirring was done during the extraction. After filtration through filter paper (Watman No. 41) the filtrate was used for antibacterial assay.

Preparation of hexane extract

The seeds were shade dried at room temperature and then were milled to a fine powder in an electrical mill and stored in the dark at room temperature for further use. 10 g of dried powdered seed was extracted by n-hexane (200 ml) (Merck, Germany) using a soxhlet apparatus (70°C, 4 h) to obtain fatty acids and the other non-polar compounds. The hexane extract was concentrated below 40°C by a rotary evaporator (Heidolph, Germany).

Methylation of hexane extract

After removing hexane using rotary evaporator, the oily mixture was derived to their methyl esters by the International Olive Oil Council (IOOC, 2001) reports by trans-esterification process. In this process, dried hexane extract was dissolved in hexane and then extracted with 2 M methanolic KOH at room temperature for 1 min. The upper phase which included fatty acid methyl esters and other non-polar compounds was analyzed by GC and GC/MS systems.

GC analyses

Gas chromatograph (GC) analyses were performed on a Shimadzu 15 A GC equipped with a split/split less injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 ml/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. The relative percentages of the characterized components are given in Table 1 (essential oil) and Table 2 (hexane extract).

GC/MS analyses

GC/MS analyses were performed using a Hewlett-Packard 5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of Heliumas carrier gas was 1 ml/min mass spectrometer (MS) were taken at 70 ev.

Identification of constituents

The constituents in the essential oil were identified by comparing their retention indices (RI) relative to n-alkanes (C_9-C_{22}), computer matching with the Wiley library and confirmed by comparing their mass spectra with those of authentic samples (Adams, 2000) or with data already available in the literature. The fatty acid methyl esters were identified by comparing their retention times and mass peaks with those of standard methyl ester mixtures and by NIST-Wiley library data search. Relative percentage amounts for both

Table 1. Essential oil composition of the leaves of *Sesbania punicea*.

Compound	[a]RI	Percentage (%)
α - Pinene	939	7.30
Camphene	953	0.08
β - Pinene	980	0.85
δ - 2- Carene	1007	0.15
1,8 – Cineole	1033	47.58
γ - Terpinene	1062	0.22
Terpinolene	1088	0.05
n - Nonanal	1098	0.30
endo-Fenchol	1112	0.06
trans-Pinocarveol	1139	0.09
Terpinen – 4 – ol	1177	0.49
α-Terpineol	1189	1.06
Trans-Carveol	1217	0.11
Cis-Myrtanol	1252	0.08
Trideane	1299	0.06
Methyl Geranate	1323	0.28
Benzyl Butyrate	1345	0.08
Tetradecane	1399	0.22
Armoadendrene	1439	0.08
Geranyl acetone	1453	0.59
Ionone	1485	1.33
δ - Cadinene	1524	0.11
Dodecanioc acid	1568	0.17
Caryphyllene oxide	1581	0.12
n - Hexadecane	1600	0.40
10-epi- γ -Eudesmol	1619	0.09
n – Penptadecane	1700	0.31
n – Octadecane	1800	0.38
Cyclohexadecane	1879	0.11
Nonadecane	1900	0.22
Farnesylaceton	1916	0.26
Phytol	1947	0.07
Henicosane	2100	11.93
Docosane	2200	0.20
Tricosane	2300	0.35
Tetracosane	2400	0.88
Pentacosane	2500	1.61
Bis(2-ethylhexyl) phetalat	2550	5.82
Hexacosane	2600	2.08
Heptacosan	2903	4.36
		91.35

[a]RI = Relative retention indices as determined onHP5-MS column using the Homologous of n-alkanes

essential oil components and fatty acid methyl esters were calculated from peak area using a Shimadzu C-R4A Chromatopac without the use of correction factors.

Antibacterial activity

The ethyl acetate extract of *S. punicea* leaves were tested against 4

Table 2. Chemical composition (%) of the hexanoic extract from the seeds of *Sesbania punicea.*

*Compound (related fatty acids)	Rt (min)	Percentage (%)
1,2-dimethyl- Benzene	5.17	0.37
Nonane	5.27	1.34
4-ethyl Octane	6.23	0.82
5-methyl Nonane	6.31	0.81
3-methyl Nonane	6.50	1.30
1-hexyl-3-methyl Cyclopentane	6.78	0.79
1-ethyl-3-methyl Cyclopentane	6.80	0.71
1,2,4-trimethyl benzene	6.90	0.64
Decane	7.00	16.63
2-ethyl-1-Hexanol	7.46	4.25
Indene	7.62	1.54
Tricyclo[5,2,1,0(2,6)]dec-3-ene	8.18	6.54
Methyl dicyclopentadiene	8.47	0.41
Tricyclo[5,2,1,0(2,6)]dec-4-ene	8.52	3.01
Undecane	8.64	0.57
Azulene	10.01	4.37
Dodecane	10.18	3.97
Tridecane	11.62	0.54
4-methyl tridecane	12.43	0.45
2-methyl Tridecane	12.50	1.15
3-methyl Tridecane	12.59	1.24
2,6,10-trimethyl Dodecane	12.68	2.11
Cyclotetradecane	12.90	1.06
Tetradecane	12.99	9.21
4.8-dimethyl tridecane	13.04	0.97
1,7-dimethyl Naphthalene	13.15	1.32
1,5-dimethyl Naphthalene	13.35	1.37
1,6-dimethyl Naphthalene	13.39	2.21
3-methyl tetradecane	13.89	0.73
Pentadecane	14.26	4.69
Hexadecane	15.47	1.41
Octadecane	17.70	0.41
Hexadecanoic acid, methyl ester (Palmitic acid)	19.00	2.20
9,12-Octadecadienoic acid, methyl ester (Linoleic acid)	20.65	6.08
9-Octadecenoic acid, methyl ester(Oleic acid)	20.70	2.65
Octadecanoic acid, methyl ester(Stearic acid)	20.93	0.66
1,2-benzene dicarboxylic acid, methyl ester	21.59	0.37
Total		88.90

*The composition of the extracts was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data and also from its retention times(Rt).

gr(+) bacteria: *Stereptococcus agalactiae*, *Stereptococcus mutans*, *Staphylococcus saprophytycous, Staphylococcus aureus* and 4 gr(-) bacteria: *Salmonella typhi, Salmonella para typhi* B*, Shigella phlexneri, Esheichia coli.* Microorganisms were identified by Research Center of Biotechnology and Industrial Center of Fungi and Bacteria collections, Iran. The *in vitro* antibacterial activity was evaluated by the disc diffusion method (DDM) according to the standard method by Bauer et al. (1966) to assess the presence of antibacterial activities of the plant extract. A bacteria culture (which has been adjusted to 0.5 McFarland standard) was used to lawn Mueller-Hinton agar plates evenly using a sterile swab. The plates were dried for 15 min and then used for the sensitivity test. The discs which had been impregnated with the plant extract were placed on the Muller-Hinton agar surface. Each test plate comprised

Table 3. Antibacterial activity of ethyl acetate extract of the leaves of *Sesbania punicea.*

Microorganism	PTCC	Gr(+/-)	Zone of inhibition (mm)*			
			Ethyl acetate extract	Antibiotics		
				C**	P**	A**
Salmonella paratyphi B	1231	-	24	22	[b]NT	NT
Stereptococcus agalactiae	1768	+	14	25	22	NT
Staphylococcus saprophytycus	1440	+	16	NT	28	NT
Salmonella typhi	1609	-	14	19	NT	NT
Shigella phlexneri	1234	-	11	NT	NT	26
Stereptococcus mutans	1683	+	[a]NA	22	26	NT
Staphylococcus aureus	1431	+	NA	NT	30	27
Escherichia coli	1395	-	NA	NT	NT	15

*Inhibition zone diameter (mm), **C: Chloramphenicol; **P: Penicilline; **A: Ampicilline; [a]NA: not active; [b]NT: not tested, PTCC: Persian type culture collection.

of 3 discs, one positive control, which is a standard antibiotic disc, one negative control and one treated disc. The standard antibiotics were chloramphenicol, penicillin G and ampicillin (10 µg) (Hindi Co., India) and the negative control was ethyl acetate (Merck, Germany). The plates were then incubated at 37°C for 24 h. After incubation, the growth inhibition zones were measured. Each test was carried out in duplicate and the average was calculated for inhibition zone diameters.

RESULTS AND DISCUSSION

The results obtained in the GC and GC/MS analyses of essential oil of leaves and hexane extract of the seeds of *S. punicea* are listed in Tables 1 and 2, respectively. Forty components comprising 91.35% of the essential oil were identified (Table 1). The oil was dominated by monoterpenes (60.60%) from which 1,8-cineole (47.58%) and α-pinene (7.30%) were the main components. Non terpenoids including hydrocarbons (23.11%), esters (5.9%) and aldehyde (0.3%) were also found in the oil, while sesquiterpenes were found too low (0.73%) in the essential oil. Essential oil composition of the aerial part of *Sesbania* species has not been investigated before and we did not find any report in the literature.

In analysis of hexane extract from the seeds of *S. punicea*, thirty seven components (88.90%) were identified from which fatty acid methyl esters (11.96%) including unsaturated fatty acids (UFAs): linoleic acid (ω-6) (6.08%), oleic acid (2.65%) and 1,2-benzene dicarboxylic acid (0.37%) were predominated to saturated fatty acids (SFAs): palmitic acid (2.20%) and stearic acid (0.66%). Hydrocarbons (77.32%) were the main components in the hexane extract of *S. punicea* seed. Seeds of the plants have always been the subject of projects in phytochemistry field (Faroog et al., 1954; Pokharkar et al., 2008; Arekemase et al., 2013). Investigations of natural products isolated from seeds have resulted in a remarkable variety of compounds having unusual structures (Powell, 2009). Seeds of many species contain uncommon fatty acids and lipids, some of which have found applications in the cosmetic industry or as renewable (non-petroleum based) industrial raw materials. In addition to protein and energy, storage substances such as carbohydrates and lipids, seeds generally contain or have the ability to produce protective compounds that are active as plant growth regulators (Meudt, 1983), fungicides (Abad et al., 2007), insecticides (Jbilou et al., 2006) and repellants of herbivores (Degenhardt, 2009). Previous study on fatty acid composition of *S. aegyptica* seed, as determined by the thiocyanometric and fractionation methods, showed oleic, linoleic, linolenic, palmitic, stearic and lignoceric acids (Faroog et al., 1954). Quantitative evaluation of the nutritional constituents of *S. sesban* seeds has been studied and the most important part of the seed were carbohydrate, protein, fiber and moisture and the results obtained from vitamin analysis revealed that the seeds of *S. sesban* are excellent sources of B vitamins and vitamin E (Arekemase et al., 2013).

In the next part of our research, we studied anti-bacterial activity of ethyl acetate extract (0.167 mg/ml) of *S. punicea* leaves aginst 8 gr(+/-) bacteria. The results presented in Table 3 showed that the extract exhibited strong inhibition activity against the gram-negative bacteria: *S. parathyphi* B (Inhibition zone diameter 24 mm), even more than chloramphenicol (Inhibition zone diameter 22 mm) as a standard antibiotic. The extract also showed moderate antibacterial activity against two gram-positive: *Streptococous* B *and Staphylococcus saprophyticus* and two gram-negative bacteria: *Salmonella typhi* and *Shigella flexneri*.

Biological activities of the leaves, flowers and bark

extracts of *Sesbania* species have been studied before. The seasonal variation of alkaloids has been investigated in leaf, bark and wood of *S. rostrata, S. exaltata* and *S. sesban*, which are medicinally important. The leaves of *S. rostrata* showed high level of lipid and alkaloid contents more than the other two species (Kadam et al., 2013). Novel chemical constituents, isolated from the leaves of *S. aculeuta* showed anti-inflammatory activity (Sharma et al., 2014). The antibacterial activity of fatty acid methyl esters from synthesis of *S. rostrata* seed by *in situ* trans esterification reaction was evaluated and the results indicated that the fatty acid methyl esters of *S. rostrara* seed was too active against the gram-negative microorganism *Pseudomonas pseudoalcaligenes* (Pokharkar et al., 2008). Anti-microbial activity of the crude extract of *S. grandifolia* flower polyphenol extract has been studied and the gram-positive bacterium, Staphylococcus aureus was reported as the most sensitive microorganism (China et al., 2012; Krasaekoopt and Kongkarnchanatip, 2005).

Conclusion

As the result, the high amount of 1,8- cineole in the essential oil of the leaves can make the plant a good natural source of this compound. The low amount of fatty acids indicates that the seed has no significant nutritional capacity. Ethyl acetate extract of the leaves showed strong antibacterial activity especially on gram-negative bacteria. The gram-negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics, so the plant can be potent for antibacterial activity against the gram-negative bacteria: *Salmonella parathyphyi* B. Further studies are needed to confirm the *in vivo* antibacterial activity and subsequent isolation and chemical characterization of the active molecules.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Ziba Jamzad (Research Institute of forests & Rangelands, Tehran, Iran) for identifying the plant material.

Conflict of Interest

Authors have not declared any conflict of interest.

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