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Chemical Composition and Some Allelopathic Aspects of Essential Oils of (*Prangos ferulacea* L.) Lindl at Different Stages of Growth

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ABSTRACT

***Prangos ferulacea* (Apiaceae) is a perennial herb with a distribution from East Europe to Middle East and Central Asia. The plant's leaves are used as animal fodder. Its fruits and roots possess biological traits that provide it with the potential to be used for medicinal purposes. The essential oils obtained through hydrodistillation from aerial parts of *Prangos ferulacea* at the vegetative and flowering stages were analyzed through GC and GC-MS. Thirty-one *vs.* seven compounds were identified in the vegetative and flowering stages' oils, respectively. While the essential oil of aerial parts at vegetative stage was dominated by α- pinene (57%), the oil at flowering stage was characterized by (E)-anethol (95.5%). The latter exhibited significant phytotoxic and fungitoxic effects in lettuce and *against Sclerotinia sclerotiorum*, respectively.**

Keywords: Allelopathy, α-pinene, (E)-Anethol, Prangos ferulacea, Sclerotinia sclerotiorum.

INTRODUCTION

Plants produce a variety of volatile, lipophilic substances known as essential oils that consist of mainly hydrocarbons or monofunctional compounds derived from metabolism of mono and sesquiterpenes, phenylpropanoids, amino as well as fatty acids (Berger, 2007). Although these volatile constituents are thought to be end products of metabolism, it has become evident that they may have some ecological roles in plants. The compounds may play an attractant role for insects in pollination and in the seed dispersing process (Tzakou *et al.,* 2004). A wide variety of essential oils are known to possess antifungal and antibacterial properties, serving as chemical defense agents against plant pathogens (Rasooli *et al.,* 2002; Razavi *et al.,* 2010b). Some essential oils exhibit cytotoxic effects being able to act as anti herbivorous agents (Erler and Tunc, 2005). In arid and semi-arid regions during the summer time, volatile compounds were found to be released from the dominant plants, suppressing the growth of herbaceous ones around them that tended to compete with the dominant plants in water and nutrients uptake (Razavi *et al.,* 2009). Thus, it becomes evident that the essential oils could serve as allelopathic agents in plants. This potential of plant volatile compounds is worth attention from an ecological point of view and could be decisive in plant diversity, abundance, dominance as well as productivity (Reigosa *et al.,* 2006). Allelopathy is also regarded as a mechanism of plant interference in agro ecosystems that offers an opportunity to manage weeds in crop rotation. It could, on the other hand adversely affect crop's yields and influence the choice of rotation (Asghari and Tewari, 2007). It has also previously been shown that allochemicals produced by plants vary depending on their growth stages and habitats (Berger, 2007).

In a previous study, the essential oil composition of *Prangos ferulacea* (L) Lindl

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fruits and umbels was described. In that investigation it was revealed that the plant fruit oil was enriched with alpha-pinene and exhibited high antibacterial activity against gram-positive bacteria (Razavi *et al.,* 2010b). In the present study the chemical composition and allelopathic activity of the essential oils in *Prangos ferulacea* leaves at vegetative and flowering stages are investigated. The plant belongs to Apiaceae and is the most widespread species of the genus. *Prangos ferulacea* is distributed from Eastern Europe to Middle East and Central Asia. It is a perennial herb that reaches 150 cm in length (Davis, 1972). The plant's leaves are used as animal fodder with its fruits and roots since possessing some special biological properties fit to be used for some special medicinal purposes.

MATERIALS AND METHODS

Plant Materials

Aerial parts of *P. ferulacea* at vegetative and flowering stages were collected from Neshagh (Miyane, East Azarbaijan Province, Iran) at an elevation of 1,726 m a.s.l. in May and July 2005. The voucher specimen (No: 1389-1) has been deposited in the herbarium of the Faculty of Sciences, Mohagheghe-Ardabili University, Ardabil, Iran.

Essential Oil Extraction

The oils from the leaves of plant at vegetative and flowering stages were isolated through hydrodistillation for 3 hours, using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulfate and stored at 4°C in the dark until being tested and analyzed (Razavi and Nejad-Ebrahimi, 2009).

Essential Oil Analysis

The oils were analyzed through GC-MS. The analysis was carried out on a Thermoquest-Finnigan Trace GC/MS instrument equipped with a DB-5 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 µm). The oven temperature was programmed to increase from 60 to 250ºC at a rate of 4 ºC min^{-1} and finally held for 10 minutes. Transfer line temperature was 250ºC. Helium was used as the carrier gas at a flow rate of 1.1 ml min^{-1} with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over the 35-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 µA.

GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 µm). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml min^{-1}, the split ratio same as GC/MS. The oven temperature was raised from 60 to 250ºC at a rate of 4ºC/min and held for 10 minutes. The injector and detector (FID) temperatures were kept at 250 and 280ºC, respectively. Semi-quantitative data was obtained from FID area percentages without the use of correction factors**.**

Identification of Essential Oil Components

Retention indices were calculated by using retention times of *n*-alkanes (C_6-C_{24}) that were injected after the oil and at the same temperature and conditions. Compounds were identified by comparison of their retention indices (RI) with those reported in the literatures (Adams, 2007) and their mass spectrum with the Wiley library (Wiley 7.0).

Phytotoxic Assay

Phytotoxic assay was carried out through Lettuce (*Lactuca sativa* L. cv Varamin) seeds while evaluating the response of seed germination, shoot and root elongation of seedlings to different concentrations of the essential oils. The oils were dispersed as an emulsion in water using Tween 20. Four

doses of the oils (0.004, 0.04 and 0.4 mg mL^{-1}) were obtained by dilution of the emulsions with deionized water (Tzakou *et al,* 2006). Seeds were surface sterilized with sodium hypo chloride (1%). Four replicates, each comprised of 25 seeds, were prepared for each treatment using sterile Petri dishes (90 mm) lined with a single sterile filter paper (Whatman, number 2). Five ml of different doses of the oils was added to each Petri dish. Prepared plates were then placed in a germination cabinet at 25°C in the dark. After 1 week, in each treatment, germination percentage was determined, root and shoot lengths measured (Razavi *et al.,* 2009).

Fungitoxic Assay

Sclerotinia sclerotiorum (Lib.) De Bary which caused stem rot disease of soybean was isolated from a soybean stem. Potato Dextrose Agar (PDA) was the medium used. Pure culture of the fungal isolate was maintained by a septic transfer to a freshly prepared PDA medium. The fungitoxic effects of essential oils were tested by growing the fungi on the PDA medium containing 1 ml of 0.01, 0.1 and 1 mg ml^{-1} of each essential oil, separately spread on the surface of the sterilized PDA Petri-dish. A dish of 4mm diameter (using a sterile core-borer) each pure culture of isolated fungi was placed on the thin film formed on the PDA just at point of intersection of two lines at the bottom of each Petri-dish. The controls contained distilled water in place of essential oils. The treatments as well as control were incubated for seven days at room temperature. The diameter of the radial growth of the fungi was assessed at the end of incubation period and then used to determine the percentage inhibition of each essential oil while using the formula:

Mycelia growth inhibition (%)= *[(dc-dt)/dc]×100(%)*

Where, *dc*= Average diameter of fungal colony in the control and *dt* = Average diameter of fungal colony in the treatment group (Razavi *et al.,* 2010a).

Statistical Analyses

Means and standard errors were calculated for germination percentages and for embryo lengths of the seedlings. Data from this study were analyzed using SPSS 11.5. Following an analysis of variance, Duncan's multiple range test was used to detect significant differences among the treatments at significance levels of $P \leq 0.05$.

RESULTS AND DISCUSSION

Aerial parts of *Prangos ferulacea* (L) Lindl yielded 0.4 and 0.2 % of yellowish color *vs.* pale olive color oil at vegetative and flowering stages, respectively. The oil at vegetative stage was comprised of thirty-one compounds representing 98% of the total oil. Seven compounds were detected from aerial parts' oil at flowering stage representing 100 % of the oil. The identified constituents of the essential oils are shown in Table 1. As could be seen from the table, the oil of plant aerial parts at vegetative stage contain alpha-pinene (57%) as the major compound. The oil at flowering stage was dominated by E-anethol (95.5%).

Table 2 presents compound class of the oils. Whereas, the oil at the vegetative stage was characterized by monoterpene hydrocarbons (72.7%), the oil plant at the flowering stage was rich in aromatic compounds (95.5%).

According to the phytotoxic assay results presented in Table 3, the leaf extracted oil of *P. ferulacea* at flowering stage demonstrated a high phytotoxic activity in lettuce. The oil stunted the root growth of lettuce with an IC_{50} value of 244.19 mg m^{-1}. The oil extracted at the vegetative stage did not exhibit considerable phytotoxic activity during the assay involving lettuce.

The fungitoxic assay of the tested oils showed the oil extracted at the flowering stage displaying fungicidal properties as compared to control. The oil reduced radical growth of *Sclerotinia sclerotiorum* mycelia

Table 1. Chemical composition of the essential oils of aerial parts of *Prangos ferulacea* at vegetative and flowering stages.

No.	Compounds	Percentage		RI[a]
		V[b]	F[c]	
1	α-Tujene	0.1	-	925
2	α-Pinene	57.0	1.2	939
3	Camphene	0.3	-	950
4	Thuja-2,4-diene	0.7	-	959
5	Sabinene	2.2	-	975
6	β- Pinene	4.5	-	983
7	Myrcene	0.9	-	989
8	Δ- 3- Carene	2.3	-	1012
9	p- Cymene	1.3	-	1017
10	Limonene	1.8	0.9	1029
11	β- Ocimene	2.9	-	1040
11	γ- Terpinene	0.1	-	1050
12	3-Ethylidene-2-methyl-1-hexen-4-yne	5.3	-	1070
13	Trans- Dihydrocarveol	0.7	-	1089
14	α- Camphene aldehyde	2.2	-	1120
15	p- Mentha-1,5-dien-8-ol	0.3	-	1130
16	Myrtenal	-	0.3	1172
17	Pinocarvone	1.3	-	1145
18	Terpin-4-ol	0.2	-	1163
19	p-Cymene-8-ol	0.1	-	1175
20	β-Ocimene	-	0.5	1220
21	E-anethole	3.9	95.5	1240
22	Thymol	0.8	-	1280
23	α-Cedrene	0.1	-	1420
24	β-Caryophyllene	0.5	0.6	1426
25	E-β-Farnesene	1.1	-	1443
26	Curcumene	0.7	-	1480
27	β-Ionone	0.1	-	1494
28	Dihydroagarofuran	0.1	-	1509
29	Kessane	1.7	-	1541
30	Spathulenol	1.2	-	1575
31	Caryophyllen oxide	3.5	-	1580
32	Humulene epoxide	0.1	-	1615
33	Tetracosane	-	0.9	2398

[a] Retention Indices; [b] Vegetative stage, [c] Flowering stage.

Table 2. Chemical class distribution of the essential oil components present in the aerial parts of. *P. ferulacea* at two growth stages.

Compound class	Percentage	
	Vegetative stage	Flowering stage
Monoterpene hydrocarbons	72.7	2.6
Oxyganated monoterpenes	0.8	0.3
Sesquiterpene hydrocarbons	2.5	0.6
Oxyganated Sesquiterpenes	6.4	-
Aromatic compounds	6.0	95.5
Ketones	1.3	-
Alchols	0.6	-
Aldehydes	2.2	-
Others	5.5	0.9

Table 3. Phytotoxic activity of essential oils extracted from aerial parts of *P. ferulacea* at vegetative (V) and flowering (F) stages.

Concentration (mg mL^{-1})	Seed germination (%)		Shoot length (mm)		Radicle length (mm)	
	V	F	V	F	V	F
0	95.0 ±1.0 ^{a}	95.0±1.0 ^{a}	17.0 ±2.3 ^{a}	17.9 ± 6.3 ^{a}	36.4 ± 2.7 ^{a}	36.2 ± 2.8 ^{a}
0.004	81.0 ±2.5 ^{a}	96.0 ± 1.6 ^{a}	16.7 ±0.7 ^{a}	17.0 ±1.9 ^{a}	24.7 ± 5.0 ^{a}	34.4 ± 5.3 ^{a}
0.04	88.0 ±2.3 ^{ab}	97.0±1.0 ^{a}	17.1±1.6^{a}	16.1 ± 1.7 ^{a}	27.4 ± 5.8 ^{a}	16.1 ± 1.7 ^{b}
0.4	83.0 ±5.2 ^{b}	91.0 ±5.2 ^{a}	8.8±1.0 ^{b}	8.7 ± 0.1 ^{b}	23.6 ± 1.2 ^{b}	13.2 ± 3.0 ^{b}

Mean values in the same column followed by the same letter are not significantly different at the 0.05 level according to Duncan test.

at doses higher than 0.01 mg m^{-1}. Whereas the radial growth of the fungi mycelia in control completely covered the Petri dishes by the 7 th day of incubation (45mm), the mycelial growth being limited to 20-25 mm in Petri dishes containing different doses of the essential oil (Figure 1).

There are many reports on chemical constituents of essential oil of different parts of *P. ferulacea* from various locations (Baser *et al.,* 1996; Sefidkon *et al.,* 1998; Amiri, 2007; Razavi *et al.,* 2010b). These reports suggest α-pinene and β-pinene as the dominant compounds in the different plant parts, although there were some differences observed in the essential oil profiles in different habitats.

Our findings tend to indicate that the essential oil quality in *P. ferulacea* leaves is remarkably different depending upon its various growth stages. E-anethole that is dominated at the flowering stage (as high as 95.5% of the oil), was found in low quantities at the vegetative stage.

It is stated in literature that anethole displays different biological activities. It is found to have antiproliferative and mosquitocidal activity (Rahman, 2000; Berger, 2007). Anethole was also identified as the most active fumigant against all pest species on the basis of activity at a given dose and exposure period (Erler and Tunc, 2005). Therefore, it is assumed that fungitoxic and phytotoxic effects of *P. ferulacea* leaves at flowering stage might be attributed to the presence of anethole.

According to the author's previous work, essential oils of the plant umbels and fruits were dominated by α-pinene. α-Pinene, beside other monoterpenes, play an attractant role for insects during pollination and seed dispersal period. It is suggested that biosynthetic pathways shift to produce some such allochemicals like anethole during the reproductive stage. This change may protect a plant against herbivorous insects.

In conclusion, the results indicate that the essential oil in aerial parts of *P. ferulacea* (at flowering stage) has a potential to combat

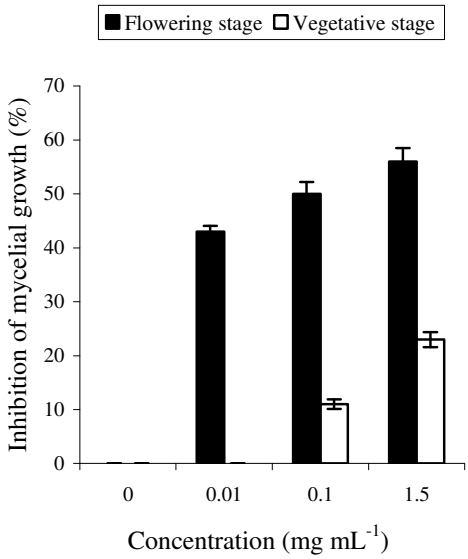

Figure1. Effects of different doses of the essential oils of *P. ferulacea* aerial parts at vegetative and flowering stage on mycelial growth of *Sclerotinia sclerotiorum.*

Sclerotinia sclerotiorum, a plant pathogen fungus causing root rot in wild plants as well as in crops. It also exhibits a considerable phytotoxic activity causing the plant to be able to stunt the growth of the competing plants in its surroundings.

Plant allelochemicals can be utilized in managing weeds, pathogens, diseases, insects as well as nematodes. The use of synthetic herbicides, fungicides and insecticides is believed to negatively affect the environment. They actually do not present the appropriate means of control as regards pathogens' resistance either. The findings of the present work revealed that *P. ferulacea* essential oils possess some allelopathic aspects that can cause them to be regarded as allelochemicals. Allelopathy studies offer a challenge in providing new concepts on an integrated new generation of such natural phytotoxines as herbicides, fungicides or insecticides and also new resistant crop varieties. Allelopathy phenomenon also bears ecological importance in natural ecosystems. It plays an important role in the determination of plant diversity, plant dominance and sociability, succession and climax of natural vegetation (Reigosa *et al.,* 2006). Further investigations are needed to elucidate the actual allelopathic potential of the *P. ferulacea* oils when the plant produced in real farming conditions.

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بررسی قابلیت آللوپا تیک و ترکیبات شیمیایی اسانس گیاه جاشیر(*Prangos ferulaceae*) در مراحل مختلف رشد

س. م. رضوی

چکیده

گیاه جاشیر (*Prangos ferulaceae*) از تیره چتریان، گیاهی است پایا به طول حداکثر ۱۵۰ سانتی متر که از اروپای شرقی تا خاور میانه و آسیای میانه انتشار دارد. برگهای این گیاه به عنوان علوفه مورد استفاده بوده و ریشه و میوه های گیاه با داشتن اثرات بیولوژیک فراوان مورد استفاده دارویی دارد. در این پژوهش، اسانس بدست آمده از اندامهای هوایی گیاه با روش تقطیر در آب، در دو مرحله رویشی و زایشی گیاه با تکنیکهای GC و GC-MS مورد آنالیز قرار گرفت. از اسانس مرحله رویشی گیاه۳۱ ترکیب و از مرحله زایشی آن ۷ ترکیب مورد شناسایی قرار گرفت. در حالی که اسانس مرحله رویشی گیاه دارای ترکیب شاخص آلفا پینن (۵۷٪) می باشد، اسانس گیاه در مرحله زایشی به طور عمده از

ترکیب آنتول به میزان ٪۹۵/۵ تشکیل شده است. نتایج بدست آمده همچنین نشان داد که اسانس گیاه در مرحله زایشی دارای اثرات بارز فیتوتوکسیک بوده و نیز اثر مهار کنندگی بر روی قارچ بیماریزای *Sclerotinia sclerotiorum* دارد. در مجموع می توان نتیجه گیری نمود که گیاه جاشیر قابلیت قابل توجه آللوپاتیک داشته و می تواند از رشد گیاهان مجاور رقیب و نیز میکروارگانیسمهای بیماریزا ممانعت نماید.