

GC-MS Analysis and Allelopathic Assessment of Aqueous Extract of *Alhagi graecorum* Boiss. Collected from Aljouf, KSA.

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ABSTRACT

In the current work, the experimental design aimed to assess the allelopathic effect of *Alhagi graecorum* Boiss. and to reveal bioactive metabolites that could interfere plant allelopathic interaction. Seeds of tomato, *Solanum lycopersicum* L., were used as allelopathic partner of *A. graecorum*. Averages of 46% inhibition in germination, 26.5 and 24% reductions in lengths of radicle and plumule of tomato's seeds were observed, respectively. Lower concentration (1%) of the aqueous extract was stimulatory for elongation of radicle and plumule. Whilst, the concentrations 2 and 4% were inhibitory. One way ANOVA revealed overall significant differences ($P= 000$). Post-Hoc analyses assured significant differences ($P< 000$) between all crosses when compared to controls. GC-MS analysis of dichloromethane extract of *A. graecorum* resulted in identification of 12 bioactive compounds, separated within 7.2 to 41.3 min from injection. Two major peaks and other minor peaks were detected. Allelopathic effect of *A. graecorum* could be attributed to some of these compounds. In addition, other identified compounds were already known to have medicinal contributions in many diseases and disorders. Conclusively, our results confirmed the allelopathic potential of *A. graecorum* on tomato's seeds. Additional studies on the biological activities of the identified compound are needed.

INTRODUCTION

Secondary metabolites of plants, called allelochemicals, break free in the environment and thus affecting other organisms in the ecosystem, directly or indirectly (Chou, 2006). This phenomenon is called allelopathy (Chung *et al.*, 2001; Ashrafi *et al.*, 2008; Chen *et al.*, 2008; Fageria *et al.*, 2008; Kong, 2008; Fang *et al.*, 2009; Mubeen *et al.*, 2012). Such natural allelochemicals could be used for eco-friendly approaches of weed management in crop rotation (Cheema *et al.*, 2002; Chung *et al.*, 2003; Nasir and Moinuddin, 2009; Vahedi, 2012). On the other hand, allelochemicals could be harmful to some field crops (Bertoldi *et al.*, 2012; Sohrabi *et al.*, 2014). Some allelochemicals have a crucial role in the inhibition of plant growth and germination (Younesabadi, 2005; Benyas *et al.*, 2011). Complexity, interaction and concentration of allelochemicals are considered cornerstone in understanding how allelochemicals could affect germination, growth, and development of other plants (Inderjit *et al.*, 2002; Mallik and Williams, 2005; Li *et al.*, 2011; Saleh, 2013). It has been

reported that allelochemicals change plant metabolism via influencing enzyme activity and vital growth processes of the plant (Einhellig, 2002; Weir *et al.*, 2004; D'Abrosca *et al.*, 2013).

Alhagi plant, from the family Fabaceae, is naturally distributed worldwide (ILDIS, 2002). Species of the genus occur in desert and semi-desert habitats (ILDIS, 2002). *Alhagi* plant is economically important as the fodder of grazing animals (Towhidi, 2007; Piri *et al.*, 2012), as a herb and as medicinal plant, too (Muhammad *et al.*, 2014). *Alhagi graecorum* Boiss is an endogenous species naturally grown all over Saudi Arabia (Boulose, 2009; Hassanein and Mazen, 2010). *A. graecorum* is a shrubby evergreen perennial herb. It is very much branched with rigid spiny twigs, commonly called Al-Aqool, Shouk Aljemaal and Camelthorn (Awmack and Lock, 2002; ILDIS, 2002; Hamid *et al.*, 2012). In addition to its importance as fodder and herb, *A. graecorum* has been reported as a folk plant in alternate medicine (Ullah *et al.*, 2013). Phytochemical analyses of *Alhagi* species clarified the presence of several bioactive compounds including sterols, glycosides, fatty acids (Kalhor *et al.*, 1997; Hamid *et al.*, 2012), phenolics (El-Saayed *et al.*, 1993; Singh *et al.*, 1999), vitamins, lupeol (Laghari *et al.*, 2011), alkaloids and flavonoids (Atta *et al.*, 2004; Saleh and Madany, 2014).

In particular, *A. graecorum* has been reported to be a repository of bioactive compounds. These are terpenoids, phenolics, flavonoids, alkaloids, steroids, resins and tannins (Laghari *et al.*, 2011; Saleh and Madany, 2014). In addition, crude extracts and purified allelochemicals of *A. graecorum* and other species have been pharmacologically studied. Hepatoprotective, antiproliferative, antioxidant, antimicrobial and cytotoxic activities have been reported (Batanouny, 1999; Alqasoumi *et al.*, 2008; Sulaiman, 2013; Wagay *et al.*, 2018). However, poor studies were carried on the allelopathic potential of *A. graecorum* (Saleh and Madany, 2014). Therefore, this piece of work aims to discover allelochemicals and to investigate the allelopathic potential of water extract of *A. graecorum* on tomato's seedling germination and early growth.

MATERIALS AND METHODS

Preparation of *Alhagi graecorum* Water Extract:

Plants were collected from Dawmat Aljandal province, Aljouf, Saudi Arabia. Samples were morphologically identified. The whole plants were air-dried, ground and plant powder were kept until further investigation.

Phytochemical analyses were carried out according to Gupta *et al.* (2013). Ten grams of plant powder were mixed with 100 mL distilled H₂O. To avoid fermentation and/ or microbial growth, the mixture was shaken overnight at 4 °C. The mixture was filtered through multi-layered cheesecloth and centrifuged for 30 min at 3000 g to get rid of cell debris. The supernatant was utilized as a stock solution of concentration 10% (w/v). Concentrations were prepared as required for allelopathic experiment.

Allelopathic Potential of *A. graecorum* Water Extract on Germination of Tomato:

Seeds of tomato, *Solanum lycopersicum* L., were surface-sterilized in 2.5% chlorox, washed 4 times by running tap water and finally washed by dH₂O. A total of 120 seeds were divided into 4 groups (30 seeds each), to be grown on Spent Mushroom Substrate (SMS) media. In the first group (Control: 3 replica, 10 seeds each), seeds were soaked in dH₂O for 4 h, and then cultivated in SMS containing dH₂O. In the experimental groups, seeds were soaked in 1.0, 2.0 and 4.0% (w/v) aqueous extract for 4 h, and then cultivated in SMS containing the same concentration of aqueous extract. Each group has 3 replica as previously mentioned in the case of control group. All jars were incubated in a growing chamber with controlled conditions (25 °C, 50- 70% RH and 14L: 10D photoperiod) for 15 days. Successful

germination was judged relying on emergence of 1 mm of radicle. At the end of experiment, percentages of germination were calculated. Lengths of plumule and radicle were measured in 5 seedlings which were selected, randomly.

Gas Chromatography-Mass Spectrometric (GC-MS) Analysis of *A. graecorum*:

The GC-MS analysis was carried out using Alient Hp 7880 with a column of 30 m length, with 0.25 mm internal diameter and 0.32 mm thickness. Helium gas was the carrier gas and flow rate was adjusted to 1 mL/ min. The temperature of the injector was set at 50 °C and temperature of the oven was adjusted at 10 °C/ min 50 to 200 °C, then 10 °C/ 3 min 200 to 250 °C ending with a 5 min isothermal at 280 °C. Two µl of the extract was injected in split mode as 10: 80, for GC-MS analysis. Results of GC-MS were interpreted using National Institute Standard and Technology (NIST) database. Produced mass spectra were identified using spectral libraries of NIST.

Statistical Analysis:

Means were compared by one way Analysis Of Variance (ANOVA) and Post-Hoc tests were carried out. The difference at $P < 0.05$ was considered significant. Statistical analysis has been carried out using the professional software SPSS for Windows (Ver. 20).

RESULTS AND DISCUSSION

Effect of Aqueous Extract of *A. graecorum* on Tomato's Seed Germination:

Table (1) summarizes the effect of serial concentrations of *A. graecorum* aqueous extract on percentage germination of tomato's seedlings, the lengths of radicle and plumule. In general, the inhibition of germination of tomato's seeds increases with the increase of concentration. An average of 46% inhibition of germination was observed. One way ANOVA revealed overall significant differences in the percentage of germination ($df= 4$, $F= 1326.1$, $P= 000$) due to the application of aqueous extract of *A. graecorum* on seeds of tomatoes. Post-hoc tests assured significant differences ($P < 000$) between all crosses except in the cross between mean and 2% extract ($P > 0.05$).

Effect of Aqueous Extract of *A. graecorum* on Elongation of Radicle and Plumule:

Lengths of radicle and plumule of tomato increased significantly ($P < 0.05$) at the concentration 1% when compared to controls (Table 1). The aqueous extract of *A. graecorum* significantly reduced ($P < 0.05$) the lengths of radicle and plumule of tomato's seedlings in a concentration-dependent profile. The average lengths of radicle and plumule were inhibited by 26.5 and 24%, respectively, as the aqueous extract was applied to tomato's seedlings (Table 1). One way ANOVA clarified overall significant differences in lengths of radicle ($df= 4$, $F= 1479.9$, $P= 000$) and plumule ($df= 4$, $F= 95.2$, $P= 000$) due to the application of aqueous extract of *A. graecorum*. Post-hoc tests assured significant differences ($P < 000$) between all crosses of radicle length. Meanwhile, differences between all crosses of plumule length were significant ($P < 000$) except in the cross between mean and 2% extract ($P > 0.05$).

Table (1): Allelopathic potential of *A. graecorum* water extract on germination and early growth of tomato's seedlings. The total period of the experiment is 15 days

Parameter	Control	1%	2%	4%	Mean
% germination	96± 0.58	63± 0.58	53± 0.58	41± 0.58	52.33± 0.19
Radicle length in cm	10.2± 0.06	12.5± 0.06	5.2± 0.06	4.8± 0.12	7.5± 0.12
Plumule length in cm	5.4± 0.12	6.1± 0.23	3.9± 0.06	2.3± 0.12	4.1± 0.17

GC-MS Analysis of *A. graecorum*:

Figure (1) illustrates potential peaks in the chromatogram of dichloromethane extract of the whole plant of *A. graecorum*. Table (2) clarifies the identified bioactive compounds of dichloromethane extract of *A. graecorum* as suggested by the spectral NIST libraries. Although the integration of the chromatogram resulted in 17 measurable peaks, only 12 bioactive compounds were identified. Retention time (RT) of the separated peaks ranges from 7.2 to 41.3 min. The calculated area of two peaks (Desulphosinigrin at RT 18.7 and [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester at RT 19.3) occupied more than 65% of the total area of the identified compounds (Table 2). However, the peaks of 3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)- at RT 16.3 min and Dasycarpidan-1-methanol, acetate (ester) at RT 21.6 min occupied the least area (1.3% for both).

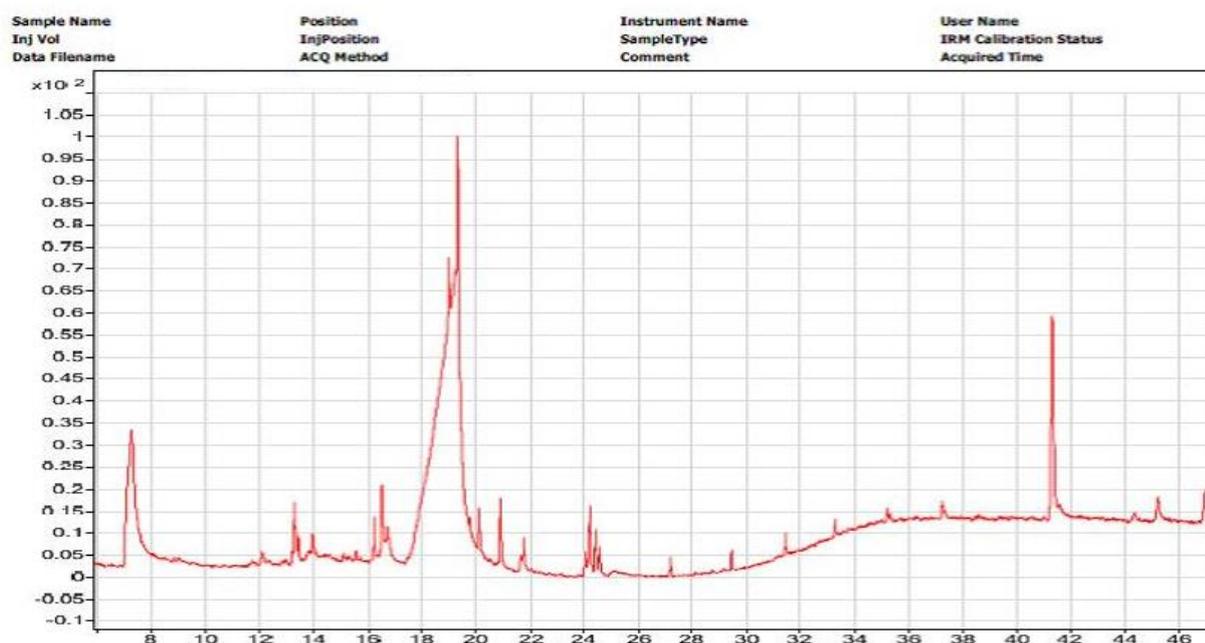


Fig. (1): Chromatogram of dichloromethane extract of *A. graecorum*, the whole plant.

Table (2): GC-MS phytochemical analysis of dichloromethane extract of *A. graecorum*, the whole plant.

No.	Compound identified	Retention time in min	Area (%)	Molecular weight (g/ mol)	Molecular formula
1	Urea, (2-ethylhexyl)-	7.257	9.8	172.157	C ₉ H ₂₀ N ₂ O
2	Astaxanthin	13.301	4.2	596.386	C ₄₀ H ₅₂ O ₄
3	3-Buten-2-one,4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)-	16.536	1.3	208.146	C ₁₃ H ₂₀ O ₂
4	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	16.751	4.1	600.490	C ₄₂ H ₆₄ O ₂
5	Desulphosinigrin	18.731	29.8	279.077	C ₁₀ H ₁₇ NO ₆ S
6	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	19.333	35.5	322.287	C ₂₁ H ₃₈ O ₂
7	6,9,12,15-Docosatetraenoic acid, methyl ester	20.114	2.3	346.287	C ₂₃ H ₃₈ O ₂
8	Cyclopropanebutanoic acid, 2- [[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl]methyl]cyclopropyl]methyl], methyl ester	20.901	2.7	374.318	C ₂₅ H ₄₂ O ₂
9	Dasycarpidan-1-methanol, acetate (ester)	21.657	1.3	326.199	C ₂₀ H ₂₆ N ₂ O ₂
10	7,10,13-Eicosatrienoic acid, methyl ester	24.203	1.8	320.271	C ₂₁ H ₃₆ O ₂
11	Cholestan-3-ol, 2-methylene-, (3β,5α)-	24.412	2.3	400.370	C ₂₈ H ₄₈ O
12	Vitamin E	41.302	4.9	430.381	C ₂₉ H ₅₀ O ₂

DISCUSSION

The present work aims to test allelopathic potential of water extract of *A. graecorum* on tomato's seedlings and to discover bioactive materials of *A. graecorum* shrub. This work describes additional evidence of the inhibitory allelopathic potential of *A. graecorum* on % germination by 46%, on radical and plumule lengths by 26.5 and 24%, respectively. Houssien *et al.* (2014) reported inhibitory effect of *A. maurorum* on arable weed and crops including *S. lycopersicum*. They found weeds with narrower leaves have been affected more than that of broader leaves. In addition, they reported phytotoxic effect of water extract of *A. maurorum* on *S. lycopersicum*. Similarly, Sadaqa *et al.* (2010) reported reduced germination percentage of onion seeds treated with shoot residues of *A. maurorum*. In addition, El-Khatib (2000) presented that aqueous extract of *A. graecorum* inhibited seed germination of *Mulva parviflora*, *Glinus lotoides* and *Chenopodium murale*. Contradictory results were presented by Saleh and Madany (2014) who documented that water extract of *A. graecorum* had no significant effect on percentage germination of seedlings of both *Vicia faba* and *Zea mays*, up to 6% concentration. Many publications have reported allelopathic potential (inhibitory and stimulatory) of different plant species on germination of seedlings (Cheema and Khaliq, 2000; Cheema *et al.*, 2000; Cheema *et al.*, 2001; El-Darier, 2002; Fikreyesus *et al.*, 2011; Al-Watban and Salama, 2012; Fahmy *et al.*, 2012; Saleh, 2013). Others reported that seed germination is less sensitive to allelochemicals than seedlings growth (Einhellig, 2004; Saleh and Madany, 2014). Our results indicated that the 1% concentration of *A. graecorum* was stimulatory to lengths of radicle and plumule of *S. lycopersicum*. Meanwhile, 2 and 4% concentrations were inhibitory. Other results were inconsistent with ours (*e. g.* Fikreyesus *et al.*, 2011; Saleh and Madany, 2014).

GC-MS analysis resulted in chromatogram with 17 peaks resolved within 7.2 to 41.3 min. Twelve bioactive compounds were identified by NIST libraries. Interestingly, a major peak of the anticancer, Desulphosinigrin, with an area of 29.8% has been identified. AntiOxidants (Astaxanthin, Vitamin E) and Antimicrobials (Cholestan-3-ol, 2-methylene-, (3 β ,5 α)-, Dasycarpidan-1-methanol, acetate ester) have been identified, too. These results suggested that many bioactive compounds of *A. graecorum* should be investigated to assure their pharmaceutical potential. Wagay *et al.* (2018) identified many bioactive from different extracts of *A. pseudalhagi*. Bioactive pharmaceuticals of *Alhagi spp.* have been reviewed, extensively (Muhammad *et al.*, 2014; Nishanbaev *et al.*, 2018). Various pharmacological and biological activities including cardiovascular, antimicrobial, anti-ulcer, antioxidant, anti-inflammatory, antipyretic, antidiarrheal, antiurolithic, depigmenting, and other activities have been reviewed (Muhammad *et al.*, 2014). In addition, many secondary metabolites have been identified from *Alhagi spp.* These metabolites could have interfered with the other species resulting in the allelopathic potential of *Alhagi*.

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ARABIC SUMMARY

التحليل الطيفي الكتلي الغازي (GC-MS)، والتقييم الأليلوباثي للمستخلص المائي لنبات العاقول *Alhagi graecorum* الذي تم تجمعه من منطقة الجوف، المملكة العربية السعودية.

هيفاء بنت عبدالعزيز الساكت الهذلول

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يهدف تصميم التجارب في هذا العمل إلى تقييم التأثير الأليلوباثي لنبات العاقول *Alhagi graecorum*، وكذلك الكشف عن المواد النشطة بيولوجيا في النبات، والتي يمكن أن تتكون سببا للتأثير الأليلوباثي لنبات العاقول. وقد استخدمت بذور الطماطم في تجربة التأثير الأليلوباثي للشوك. وقد لوحظت معدلات تثبيط بنسبة 46% في الإنبات، و تثبيط 26.5 و 24% في أطوال الجذير، و السويق الجنيني في بذور الطماطم، على التوالي. وقد كان التركيز المنخفض (1%) من المستخلص المائي محفزًا لاستطالة الجذير والسويق، بينما كانت التركيزات 2 و 4% مثبطة لنمو الجذير والسويق. وقد أثبت التحليل الإحصائي (ANOVA) وجود فروق معنوية ($P=000$)، و أكدت تحليلات مقارنة أزواج التجارب وجود فروق معنوية أيضا ($P<000$) بين نتائج جميع التركيزات عند مقارنتها بالتجربة الضابطة. وقد أسفر تحليل GC-MS لمستخلص الشوك من ثنائي كلورو الميثان عن تحديد 12 مركبا ذا نشاط حيوي، مفصولة خلال 7.2 إلى 41.3 دقيقة من بداية الحقن، و قد تم اكتشاف قمتين رئيسيتين وقمم صغيرة أخرى. و يمكن أن يعزى تأثير الأليلوباثي لنبات العاقول إلى بعض هذه المركبات. بالإضافة إلى ذلك، فإن من المعروف بالفعل أن كثيرا من المركبات التي تم فصلها لها إسهامات طبية في العديد من الأمراض والاضطرابات. و قد أكدت نتائجنا التأثير الأليلوباثي لنبات العاقول على بذور الطماطم. و لكن هناك حاجة لدراسات إضافية حول الأنشطة البيولوجية للمركبات التي تم التعرف عليها.